FOOD CONVERSION EFFICIENCY IN TRANSGENIC TILAPIA WITH ACCELERATED GROWTH

Isabel Guillén
Mammalian Cell Genetics Division.
Centro de Ingeniería Genética y Biotecnología.
P.O.Box 6162, Havana, Cuba
E-mail: jose.delafuente@cigb.edu.cu

Antonio Morales, Mario Pablo Estrada and José de la Fuente.
Mammalian Cell Genetics Division.
Centro de Ingeniería Genética y Biotecnología,
P.O.Box 6162, Havana, Cuba

Introduction

Growth is a complex process that is regulated by the interaction of different factors. The growth hormone stimulates growth in fish by producing an increase in DNA synthesis and subsequent cell division. However, the understanding of how the increased growth hormone levels interact with behavioural components like growth rate and food conversion is very limited. The search for new fish strains with accelerated growth represents an economical necessity for aquaculture development. This objective increases the need to study the mechanisms of growth in order to obtain animals with improved growth performance and/or efficiency in food conversion (Houlihan, 1993; de la Fuente, 1998a).

The selection of growth hormone (GH) genes for transgenesis has shown to produce fish strains with improved growth conditions (de la Fuente, 1998a; MacLean, 1994; de la Fuente, 1998b). Other methods based on supplying growth factors in the diet or water and injecting protein preparations are very expensive and laborious (Guillén, 1998).

We have generated a transgenic tilapia line with improved growth performance (de la Fuente, 1998b; Martínez, 1996; de la Fuente, 1998c; Estrada, 1998). This line expresses ectopically low levels of endogenous growth hormone (de la Fuente, 1998b; Martínez, 1996; Guillén, 1995; Hernández, 1997).

Here we compared the biological food conversion factor between transgenic and control (wild type) tilapia. The transgenic tilapia exhibited about 3.6 fold less food consumption than the control tilapia ($p \leq 0.001$, student-t Test). Furthermore, the biological food conversion factor was significantly ($p \leq 0.03$;Student-t Test) higher (3.4x) in the transgenic group (2.8±3) when compared to the control group (9.7±9).

These experiments demonstrate that GH-transgenic tilapia with accelerated growth are also more efficient in food conversion, thus adding additional value to this transgenic tilapia line.

Materials and Methods

Animals and husbandry

Twenty size-selected tilapias (107.26±14.1 average wet weight), supplied by Mampostón (San José, Havana, Cuba) were acclimated in 500 litters aquarium with re-circulating freshwater at 25°C with constant photoperiod (14 hours light and 10 hours darkness) and fed with commercially prepared pellets (CENPALAB, Havana, Cuba). Daily rations equivalent to 2% of the body weight were administered twice a day until they were used in the experiments.

Preparation of food pellets

Pellets labelled with glass beads were prepared by mixing the commercial food pellets with 3.7% vegetable oil, 1.8% Carboxymethyl cellulose (CMC), 9.2% powder milk, distillate water and glass beads in a ratio of 152.8±7.6 beads (0.5mm) per gram food pellets. Pellets were formed following compression of the mix through a silicon applayer with a 2mm-diameter, and dried during 24 hours at 26°C (Fig 1).

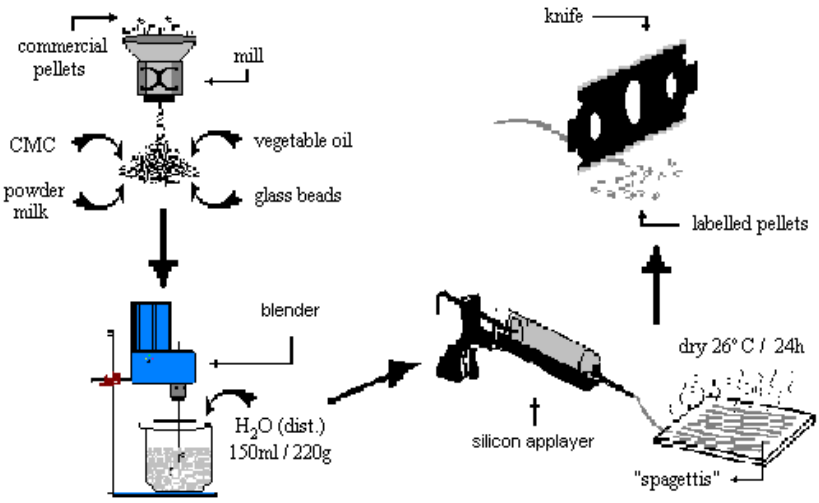


Figure 1. Schematic representation of the labelled pellets preparation.

Experimental procedure

Two experimental groups of 10 animals each, containing 7 males and 2 females (109.7±13.5g) and 6 males and 4 females (104.8±14.7g) of non-transgenic and transgenic (F2 heterozygous) tilapia respectively, were separated in aquariums of 500 L each.

The experiment was conducted during five weeks with weekly measurements of individual fish weight and food consumption using radiography. Tilapia were fed three times daily with commercial food pellets (ratio=4% total fish weight), except on the day where measurements were conducted. This day tilapia were fed once with the same ratio of food pellets labelled with glass beads to aloud X-ray diffraction.

Definition of the biological food conversion factor (BCF)

The BCF is the individual ratio between the food intake and the dry weight gain. This factor was calculated using the formula BCF=food intake/20% of dry weight gain.

Results and discussion

The results showed that food intake was dramatically different between both groups of animals (Figure 2).

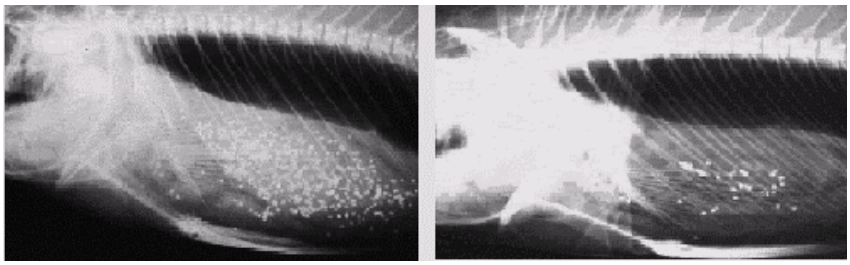


Figure 2. The picture represents a tipical radiography showing the glass beads into the stomach of a non-transgenic (left) and transgenic tilapia.

As shown in the Table 1, to achieve the same growth rates transgenic tilapia exhibited about 3.6 fold less food consumption than control non-transgenic tilapia ($p \leq 0.001$, Student-t Test). Furthermore, the BCF was significantly (3.4x) higher in the transgenic group (2.8 ± 0.63) when compared to the control group (9.7 ± 2.96) ($p \leq 0.03$; Student-t Test).

Evidences have been reported on that growth hormone improve the appetite and food conversion in vertebrates (Markett, 1977; Gill, 1985; Johnsson, 1994). However, our results showed a decrease of food intake in transgenic fish. The transgenic tilapia attained with less food a similar weight gain than the control group. This physiological and phenotypical behaviour is probably related to the direct or indirect effect that produce the growth hormone on the hypothalamic centres that affect appetite (Markett, 1977). The fact that transgenic tilapia did not growth faster than controls in this experiments was because of the experimental conditions employed.

Parameter	Transgenic tilapia	Non-transgenic tilapia
Initial weight (week 1)	104.8±5.94 g	109.7±5.9g
Final weight (week 5)	126.17±8.60g	137.5±10.7g
Total food intake	9.12±1.79g	33.0±5.2g
Dry weight gain	4.26±1.0g	5.56±1.13g
Biological food convention factor	2.85±0.63	9.7±2.96g

Table 1. Summary of the food conversion experiment in tilapias. Tilapia were weekly weighted and the food intake determined to calculate the BCF (average ± SE; n=10).

These results support the hypothesis that ectopic expression of growth hormone is an important determinant to improve food conversion efficiency in fish. Furthermore, these transgenic tilapia show an improved growth with a better food conversion efficiency, thus making this transgenic line more attractive for aquaculture.

Acknowledgment

The authors would like to thank Drs DF Houlihan (Department of Zoology, University of Aberden, UK) for encouraging us to conduct these experiments, R. Telles and his staff (Centro de Investigaciones Medico-Quirurgicas, Habana, Cuba) for excellent technical assistant during X-ray analysis and Asterio Cruz for helping with the statistical analyses. This work was partially supported by the International Centre for Genetic Engineering and Biotechnology Collaborative Research Programme (Project CRP-CUB93-05).

References

de la Fuente J. (1998a). Gene transfer in aquatic organism in the framework of modern biotechnology. In: Gene transfer in aquatic organism (J de la

Fuente and F.O.Castro, Eds.) Chapter 1. RG Landes Company: Austin, Texas and Springer Verlag: Germany. pp.:1-15.

de la Fuente, J., Martinez, R., Guillén, I., Estrada, M.P., Lleonart, R. (1998b). Gene transfer in tilapia for accelerated growth: from the laboratory to the consumer.. In: Gene transfer in aquatic organisms (J de la Fuente and F.O.Castro, Eds). Chapter 6. RG Landes Company: Austin, Texas and Springer Verlag: Germany. pp: 83-106.

de la Fuente J, Guillén I, Martínez R, Estrada MP. (1998c). Gene transfer in tilapia: laboratory and biotechnological applications. Accompanying paper

Estrada MP, Herrera F, Cabezas L et al. (1998). Culture of transgenic tilapia with accelerated growth under different intensive culture conditions. Accompanying paper

Gill JA, Sumpter JP, Donaldson EM, Dye HM, Souza L, Berg T, Wypych J AND Langley K. (1985). Recombinant chicken and bovine growth hormones accelerate growth in aquaculture juvenile Pacific salmon (*Oncorhynchus kitsutch*). Biol Technology 3(643-646).

Guillén I, Martínez R, Hernández O, Estrada MP, Pimentel R, Herrera F, Morales A, Rodríguez A, Sánchez V, Abad Z, Hidalgo Y, Lleonart R, Cruz A, Vázquez J, Sánchez T, Figueroa J, Krauskopf M, de la Fuente J. (1995). Characterization of a transgenic tilapia lines with different estopic expression of tilapia growth hormone. In aquaculture biotechnology symposium proceeding. Donalson EM and MacKinlay DD (eds) pp 63-75.

Guillén I, Lleonart R, Agramonte A, Morales R, Morales A, Hernández CA, Vazquez MM, Diaz M, Herrera MT, Alvarez-Lajonchere L, Hernández O and de la Fuente. (1998). J. Physiological changes in the juvenile euryhaline teleost, the tilapia *Oreochromis hornorum*, injected with *E. coli*-derive homologous growth hormone. Journal of Marine Biothenology 6 (in press)

Hernández O, Guillén I, Estrada MP, Cabrera E, Pimentel R, Piña JC, Abad Z, Sánchez V, Hidalgo Y, Martínez R, Lleonart R, and de la Fuente J

(1997). Characterization of transgenic tilapia lines with different ectopic expression of tilapia growth hormone. Molecular Marine Biology and Biotechnology 6(4): 364-375.

Houlihan DF, Mathers EM, and Foster A. (1993).Biochemical correlates of growth rate in fish. Fish Ecophysiology. Edited by Cliff Rankin and Frank B. Jensen. Published by Chapman & Hall, London.

Johnsson JI and Bjornsson BTH. (1994). Growth hormone increases growth rate, appetite and dominance in juvenile rainbow trout, *Oncorhynchus mykiss*. Anim Behav 48: 177-186.

MacLean N and Rahman MDA. (1994). Transgenic fish. In: MacLean, (ed) Animals with novel genes. Cambridge, UK: Cambridge University Press, pp 63-105.

Markett JR, Higgs DA, Dye HM and MacQuarrie DW (1977). Influence of bovine growth hormone on growth rate, appetite, and food conversion of yearling coho salmon (*Oncorhynchus kitsutch*) fed two diets of different composition. Can J. Zool 5: 74-83.

Martínez R, Estrada MP, Berlanga J, Guillén I, Hernández O, Cabrera E, Pimentel R, Morales R, Herrera F, Morales A, Piña JC, Abad Z, Sánchez V, Melamed P, Lleonart R, de la Fuente. (1996). Growth enhancement in trangenic tilapia by ectopic expression of tilapia growth hormone. Molecular Marine Biology and Biotechnology. 5(1): 62-70.

THE COMPETITIVE FEEDING RESPONSE OF

DIPLOID AND TRIPLOID

BROOK TROUT (*Salvelinus fontinalis*)

Robyn A. O'Keefe
Department of Biology, University of New Brunswick
Fredericton, New Brunswick, Canada E3B 6E1
Phone: (506) 453-4583 Fax : (506) 453-3583
email: okeefe@unb.ca

Tillmann J. Benfey
University of New Brunswick, Department of Biology

EXTENDED ABSTRACT ONLY DO NOT CITE

An inherent characteristic of triploids is they have larger but fewer cells, which may result in differences in aggressive behaviour compared to diploids (Benfey, 1998). The purpose of this experiment was to test the competitive feeding response of diploid and triploid brook trout. A simple method for demonstrating competitive feeding hierarchies is to feed fish limited rations, one pellet at a time, and record the number of pellets consumed. Hierarchical rank is then assigned on the basis of number of pellets consumed by each fish, with the highest ranked fish consuming the most pellets (Metcalfe et al., 1990). An experiment using this approach was designed involving 3 diploid and 3 triploid size-matched fish. These fish were placed in a v-shaped Plexiglas trough and fed half their normal ration 3 times a day for 5 days. This protocol was repeated for 12 trials with small brook trout (0.50-2.0g) and 10 trials with larger fish (19.0-33.0g).

Hierarchies developed within 1-2 days, with dominant fish in the upstream position at the point of food entry and subordinate fish swimming downstream at the end of the trough in small schools. This observation is reflected in the number of pellets eaten by each fish and their assigned rank (Figures 1 and 2). There was no difference between the average rank of diploids and triploids among the large brook trout (Figure 1, $p > 0.5$ by ANOVA). There was,

however, a difference in average rank between ploidies in the small fish, with the diploids having the higher average rank (Figure 2, $p = 0.0004$). There was also a significant ploidy-weight interaction among small fish ($p = 0.018$). When these results were analyzed separately by weight and length it was found that the difference in competitive feeding behaviour only applied to fish of the same length ($p = 0.032$). Consequently, one would expect a difference in condition factor between the ploidies but this was not the case for small brook trout (diploid 1.05 ± 0.10 [SD] and triploid 1.00 ± 0.08, $p = 0.078$), nor for large brook trout (diploid 1.35 ± 0.11 and triploid 1.31 ± 0.14, $p = 0.162$).

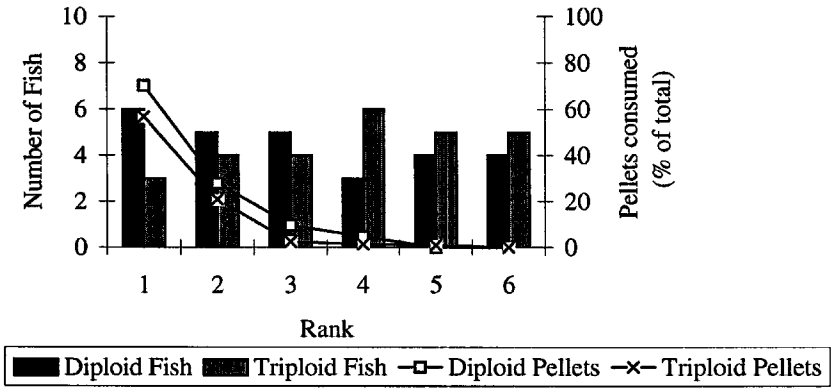


Figure 1.

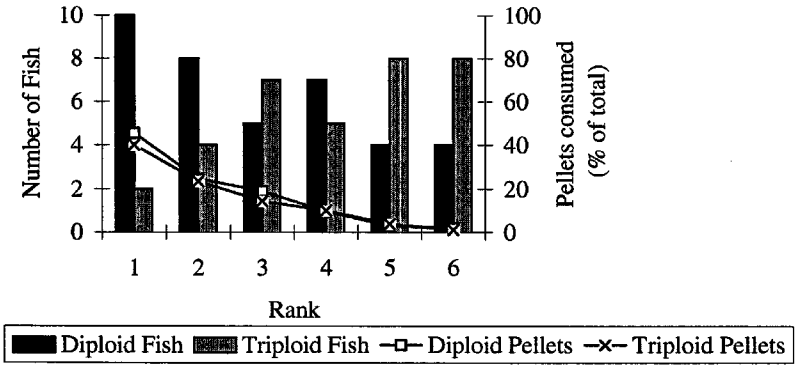


Figure 2.

Holtby et al. (1993) demonstrated with first feeding coho salmon (*Oncorhynchus kisutch*) that body morphology was a good predictor of dominance, with the deeper-bodied fish being dominant. This was not the case with our experiments since there was no difference in condition factor between the ploidies. Nevertheless, there is some literature that suggests that the initial development and growth of triploids is delayed compared to diploids but that triploids catch up quickly (McGeachy et al., 1995). There is also some evidence that aggression in fish declines with age and social experience (Francis, 1990). This may be the case in these experiments, where initially there was a difference in behaviour between the ploidies, but this diminished with time.

The experiments reported here were repeated with another strain of brook trout and with Atlantic salmon (*Salmo salar*) and the results were similar, with no difference in competitive feeding behaviour between the ploidies (O'Keefe and Benfey, 1997).

Acknowledgements

We thank R. Mureika, E. Stillwell and A.J. Wiggs for help with this project. This research was funded by a grant from the Natural Sciences and Engineering Research Council of Canada. The experimental protocol was approved by the UNB Animal Care Committee, meeting Canadian Council of Animal Care Guidelines.

References

Benfey, T.J. 1998. The physiology and behavior of triploid fishes. Rev. Fish. Sci.: in press.

Francis, R.C. 1990. Temperament in a fish: A longitudinal study of the development of individual differences in aggression and social rank in the Midas cichlid. Ethology 86:311-325.

Holtby, L.B., D.P. Swain, and G,M. Allan. 1993. Mirror-elicited agonistic behaviour and body morphology as predictors of dominance status in

juvenile coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquatic Sci. 50:676-684.

McGeachy, S.A., T.J. Benfey, and G.W. Friars. 1995. Freshwater performance of triploid Atlantic salmon (*Salmo salar*) in New Brunswick. Aquaculture 137:333-341.

Metcalfe,N.B., F.A. Huntingford, J. Thorpe and C.E. Adams. 1990. The effects of social life-history variation in juvenile salmon. Can. J. Zool. 68:2630-2636.

O'Keefe, R.A. and T.J. Benfey. 1997. The feeding response of diploid and triploid Atlantic salmon and brook trout. J. Fish Biol. 51:989-997.

THE GROWTH AND FOOD CONSUMPTION

OF DIPLOID AND TRIPLOID

BROOK TROUT (*Salvelinus fontinalis*)

MONITORED BY RADIOGRAPHY

Robyn A. O'Keefe
Department of Biology, University of New Brunswick
Fredericton, New Brunswick, Canada E3B 6E1
Phone: (506) 453-4583 Fax : (506) 453-3583 email: okeefe@unb.ca

Tillmann J. Benfey
Department of Biology, University of New Brunswick

Introduction

Radiography can be used to describe hierarchy formation within a fish population (Talbot and Higgins, 1983). The aim of this research was to use radiography to study the social order of diploid and triploid brook trout raised in separate and mixed ploidy groups. Some research suggests that diploids are more aggressive than triploids (Carter et al., 1992). It was predicted that if diploids are more dominant than triploids then they would out-compete triploids for food in a mixed ploidy environment.

Methods

Equal numbers of diploids and triploids were implanted with passively-integrated transponders (pit-tags) and randomly divided into 6 circular tanks to give duplicate tanks containing 24 diploids, 24 triploids, or 12 of each ploidy. Fish were hand fed half the manufacturer's recommended ration, delivered at a single point in the tank once per day. Daily food intake was estimated using radio-graphy. Every 3 weeks fish were fed a labeled diet containing ballotini glass beads, and then 2 hours later anaesthetized and radiographed (McCarthy et al., 1992). The day after radiography and prior to feeding, the fish were again anaesthetized and measured for weight and fork length. This procedure was

repeated 14 times over a 42-week period. The labeled diet was also radiographed to determine the relationship between dry weight of food and number of glass beads. The radiographs were used to count the number of glass beads in the gastrointestinal tract.

Group share of meal was calculated as GSM = (SM/ SM) X 100, where SM is the share of meal eaten by each fish and SM is the total amount of food consumed by the group in mg dry food. Specific growth rate was calculated as $SGR = [(\ln W_T - \ln W_t)/(T-t)] \times 100$, where W_T and W_t were fish weights at times T and t, and (T-t) was the number of days between weighings. The relative food intake of each fish was calculated as RFI = mg dry food/g wt/day. The day-to-day coefficient of variation in food intake was calculated as CV_{RFI} = (standard deviation/mean) X 100. The formation of a strong hierarchy is indicated by a strong positive correlation between GSM and SGR and a strong negative correlation between GSM and CV_{RFI} (Jobling and Baadvik, 1994).

Results and Discussion

The brook trout grew on the limited diet provided. A correlation between food intake and growth rate was apparent, with the faster growing fish consuming a higher portion of the meal (Figure 1, ANOVA). However, there was no difference between ploidies whether fish were cultured as separate or mixed ploidy groups. This indicates that triploids were competing equally well with diploids under both culture conditions. No hierarchy developed between the fish in either the separate or mixed ploidy groups, as demonstrated by the lack of correlation between GSM and both SGR and CV_{RFI} (Figure 2, ANOVA).

In order to promote hierarchy formation, the fish in each tank were fed half their normal ration and from a localized feeding point. Other researchers have found both these methods to be an effective

way of promoting the formation of hierarchies (Ryer and Olla, 1996). This was not the case in our experiments, perhaps because the quantity of food given was too high to promote competition.

Literature reporting growth rates of triploids is conflicting (Galbreath et al., 1994), and it has been suggested that diploids need to be evaluated on a species-by-species basis (Fast et al., 1995). Within this context, it may also be well

advised to examine the optimal conditions for growth of triploids, which may not be the same as for diploids.

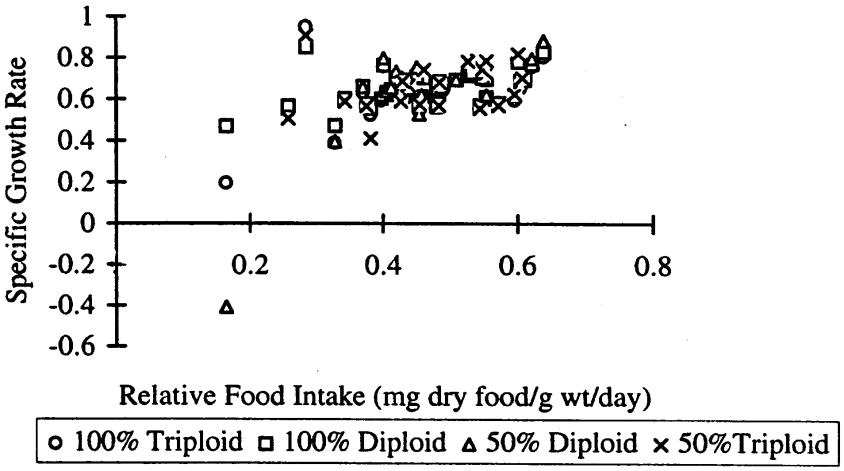


Figure 1. The relationship between specific growth rate and mean relative food intake for diploid and triploid brook trout cultured in separate and mixed ploidy groups (100% Triploid r = 0.407, p = 0.0040; 100% Diploid r = 0.440, p = 0.0017; 50% Triploid r = 0.672, p = 0.0003; 50% Diploid r = 0.823, p = 0.0001).

In conclusion, the brook trout in our experiment established stable populations in separate and mixed ploidy groups, with little variation in growth or food intake. Under limited food supply, triploids performed as well as diploids.

Acknowledgements

We thank R. Mureika, D. Peterson, K. Richards, S. Rogers, E. Stillwell, A. Wiggs, D. Sewell and the staff at City Animal Hospital for their advice and assistance with this project. This research was funded by a grant from the Natural Sciences and Engineering Research Council of Canada. The

experimental protocol was approved by the UNB Animal Care Committee, meeting Canadian Council of Animal Care Guidelines.

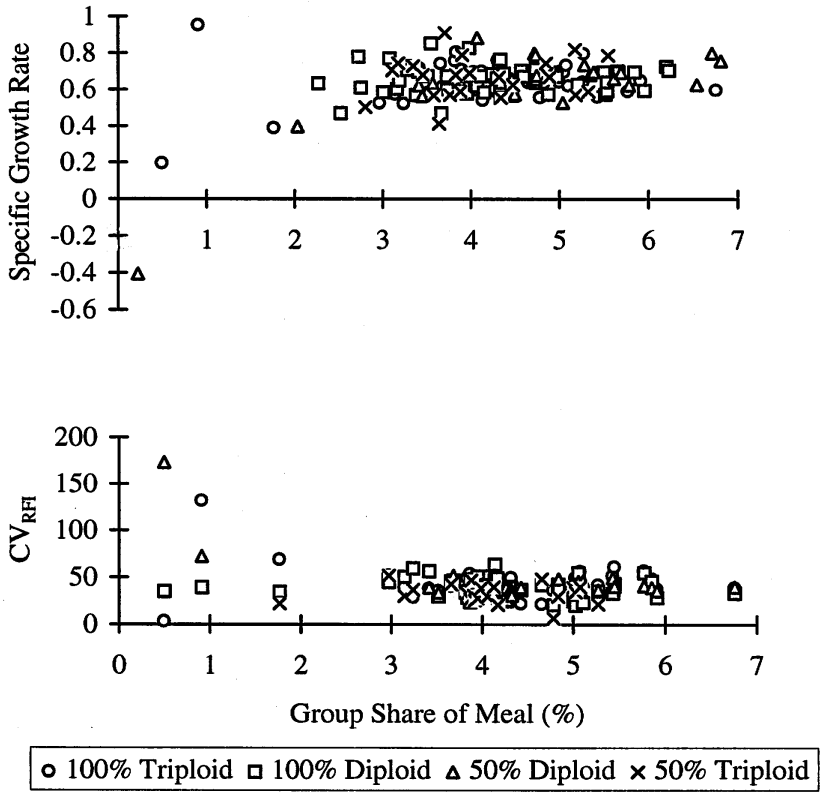


Figure 2. The relationships between group share of meal and specific growth rate (100% Triploid r = 0.118, p = 0.61; 100% Diploid r = 0.132, p = 0.37; 50% Triploid r = 0.231, p = 0.12; 50% Diploid r = 0.340, p = 0.12) and coefficient of variation for relative food intake (100% Triploid r = -0.032, p = 0.83; 100% Diploid r = -0.232, p = 0.11; 50% Triploid r = -0.087, p = 0.68; 50% Diploid r = 0.62, p = 0.77) for diploid and triploid brook trout cultured in separate and mixed ploidy groups.

References

Carter, C.G., I.D. McCarthy, D.F. Houlihan, R. Johnstone, M.V. Walsingham and I. Mitchell. 1994. Food consumption, feeding behaviour, and growth of triploid and diploid Atlantic salmon *Salmo salar* L., parr. Can. J. Zool. 72:609-617.

Fast, A.W., T. Pewnim, R. Keawtabtim, R. Saijit, F.T. Te and R. Vejaratpimol. 1995. Comparative growth of diploid and triploid Asian catfish *Clarias macrocephalus* in Thailand. J. World Aquacul. Soc. 26:390-395.

Galbreath, P.F., W. St.Jean, V. Anderson and G.H. Thorgaard. 1994. Freshwater performance of all-female diploid and triploid Atlantic salmon. Aquaculture 128:41-49.

Jobling, M. and B.M. Baardvik. 1994. The influence of environmental manipulations on inter-and intra-individual variation in food acquisition and growth performance of Arctic charr, *Salvelinus alpinus*. J. Fish Biol. 44:1069-1087.

McCarthy, I.D., C.G. Carter and D.F. Houlihan. 1992. The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Biol. 41:257-263.

Ryer, C.H. and B.L. Olla. 1996. Growth depensation and aggression in laboratory reared coho salmon: the effect of food distribution and ration size. J. Fish Biol. 48:686-694

Talbot, C. and P.J. Higgins. 1983. A radiographic method for feeding studies on fish using metallic iron powder as a marker. J. Fish Biol. 23:211-220.

EFFECTS OF DIETARY CARBOHYDRATE

ON GLUCOSE TOLERANCE AND GROWTH

OF STRIPED BASS

Brian C. Small
Dept. Animal & Avian Sciences, University of Maryland
College Park, MD 20742
Phone: 301-405-3016 Fax: 301-314-9059
Email: bs106@umail.umd.edu

Joseph H. Soares, Jr.
Dept. Animal & Avian Sciences, University of Maryland
College Park, MD

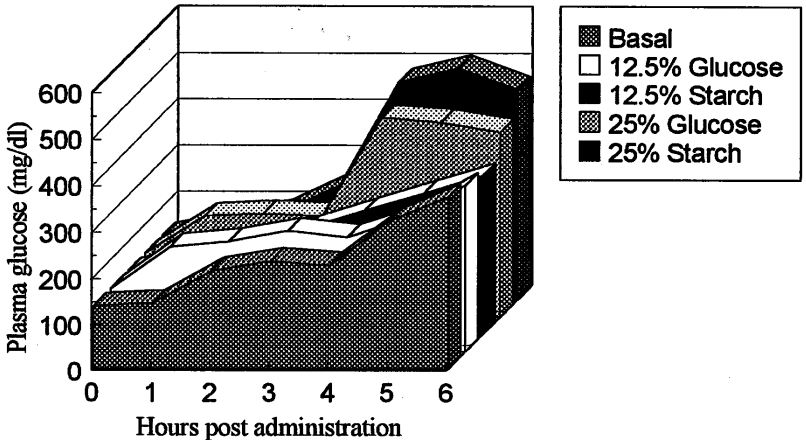
EXTENDED ABSTRACT ONLY DO NOT CITE

Continued and future development of the food fish aquaculture industry will require the development of lower cost, more efficiently utilized diets. Substituting lower cost plant feedstuffs for higher cost animal feedstuffs could potentially reduce feed costs by as much as 50 %. A common problem with feeding plant feedstuffs to fish is the high level of carbohydrate in these ingredients. Studies in a number of species indicate a wide range in the ability of fish to utilize carbohydrates. The objective of these experiments was to study carbohydrate utilization and its effect on growth performance in the striped bass, *Morone saxatilis*.

Two experiments were performed. In the fist experiment, the objective was to determine the effect of dietary carbohydrate complexity and level on glucose tolerance. Juvenile striped bass weighing approximately 76 g per fish were fed one of five dietary treatments containing either 0, 12.5 or 25 % carbohydrate as glucose or corn starch for a period of one month. After four weeks, fish were weighed to determine the quantity of glucose to be administered for the oral glucose challenge. Fish were fasted for 24 hours then orally administered 167 mg glucose/100 g body weight. Blood samples were collected at 0, 1, 2, 3, 4, 5, and 6 hours post administration from the caudal vein using heparinized syringes

for the determination of plasma glucose concentrations and glucose tolerance. Plasma glucose concentrations were determined using a Glucose (HK) reagent kit (Sigma Diagnostics, St. Louis, MO) and measured spectrophotometrically at 340 nm.

The objective of the second experiment was to determine the effect of increasing levels of dietary carbohydrate on growth performance and carbohydrate metabolism in striped bass. The experimental group was composed of 147 g juvenile striped bass fed one of five diets containing 0, 10, 15, 20 or 25 % carbohydrate as glucose for a period of two months. Parameters measured included weight gain, feed conversion ratio, hepatosomatic index (HSI), and glucose tolerance. Glucose tolerance was again measured following a 24 hour fast and an oral glucose challenge of 167 mg glucose/100 g body weight. Blood samples were collected at 0, 2, 4, 6, 8, 10, 12 and 24 hours post administration. Plasma glucose concentrations were determined as described for experiment one.



Results of the glucose tolerance data collected in experiment one after four weeks on dietary treatments of glucose or corn starch indicated a greater tolerance for dietary carbohydrate levels as high as 12.5 % but less than 25 %, irrespective of carbohydrate complexity. This is demonstrated by the steep increase in plasma glucose at four hours in the 25 % carbohydrate treatments

(Figure 1). At six hours post administration basal levels of plasma glucose were not reached in any of the treatments.

In experiment two, significant differences ($p<0.05$) were detected between 15 and 20 % carbohydrate for both weight gain and feed conversion ratio. HSI data demonstrated a significant increase ($p<0.05$) in liver size for all diets containing carbohydrate (Table 1). Glucose tolerance data in experiment two indicated a prolonged hyperglycemia across all treatments with plasma glucose levels approximating zero hour concentrations at 24 hours post administration. From these experiments it can be concluded that striped bass are able to effectively utilize both simple and complex carbohydrates at levels less than 20 % of the diet without a significant decrease in performance.

Table 1. Growth performance of striped bass after 12 weeks feeding on diets with graded levels of carbohydrate.

% Glucose	Weight gain (g)	FCR	HSI
0	88^{a}	1.17^{a}	0.90^{a}
10	87^{ab}	1.13^{a}	1.35^{b}
15	92^{a}	1.15^{a}	1.40^{b}
20	68^{c}	1.33^{b}	1.25^{b}
25	77^{bc}	1.21^{ab}	1.46^{b}
PSE	3	0.04	0.10

^{abc}Means (n=2) in the same row not sharing similar superscripts are significantly different ($P<0.05$).

THE EFFECTS OF RATION SIZE AND TEMPERATURE ON THE BIOENERGETICS OF JUVENILE CALIFORNIA STEELHEAD, *ONCORHYNCHUS MYKISS*

Christopher A. Myrick
Department of Wildlife, Fish, and Conservation Biology
University of California, Davis, CA 95616
ph: (530) 752-8659 fax: (530) 752-4154
email: camyrick@ucdavis.edu

Joseph J. Cech, Jr.
Department of Wildlife, Fish, and Conservation Biology
University of California, Davis, CA 95616
ph: (530) 752-3103 fax: (530) 752-4154
email: jjcech@ucdavis.edu

Introduction

Steelhead (*Oncorhynchus mykiss irideus*) are experiencing widespread population declines, along the Pacific Coast of North America, with only populations in remote areas of Alaska and British Columbia remaining close to historical levels. In California (CA), steelhead stocks have declined to the point where many populations are listed (USA) as threatened and endangered. The population declines in California can be attributed to a number of factors, including over-fishing, habitat loss, and poor management practices.

Now that CA steelhead have state and federal protection, efforts are underway to restore stocks. Such efforts require detailed knowledge of steelhead's ecological and physiological requirements, especially when management strategies include the use of bioenergetic modeling. The purpose of this project was to collect data on the effects of temperature and ration size on the bioenergetics and physiological performance of juvenile CA steelhead. It is hoped that this data set will provide a baseline for modeling efforts and will increase the understanding of the CA steelhead physiology and ecological tolerances and requirements.

Materials and Methods

Steelhead from the Mokelumne River State Fish Hatchery were used in a 30-d growth and food consumption experiment with 3 temperature (11, 15 and 19°C) and 2 ration (*ad libitum* and ~ 80% *ad lib.*) levels. Each temperature/ration treatment had 4 replicate tanks (25 fish/tank). Growth and food consumption was monitored and used to calculate specific growth rates (SGR) and consumption rates (SCR). At the conclusion of the growth/food consumption experiment, fish were kept under the same conditions and the following parameters were measured: resting routine oxygen consumption rates (MO_2); critical thermal tolerance (CTM); short term thermal preference using a 1.5 m gradient tank (20°C T); critical swimming velocity (U_{crit}).

Results

Actual food consumption rates for the 11, 15 and 19°C reduced-ration treatments were 79, 88 and 78%, respectively. SGR and SCR data are summarized in Table 1.

Treatment	Sample size	Specific Growth Rate (% body wt./d)	Specific consumption rate (% body wt./d)
11°C, reduced ration	4	0.74 ± 0.08†	1.36 ± 0.04
11°C, full ration	4	0.78 ± 0.07	1.66 ± 0.11*
15°C, reduced ration	4	1.13 ± 0.11	1.47 ± 0.02
15°C, full ration	4	0.99 ± 0.16	1.59 ± 0.15
19°C, reduced ration	4	1.38 ± 0.12	2.05 ± 0.05†
19°C, full ration	4	1.36 ± 0.07†	2.33 ± 0.04*†

Table 1. Summary of juvenile steelhead specific growth and consumption rates. Significant differences among ration levels at the same temperature are denoted by (*). Significant differences among temperatures at the same ration level are denoted by (†).

MO2, Ucrit, CTM and thermal preference data are summarized in Table 2.

Treatment	Final preferred temperature (°C)	Critical thermal tolerance (°C)	Oxygen consumption rate mg/h/g$^{0.66}$	Critical swimming velocity (L/s)
11°C, reduced ration	18.3 ± 0.97 (10)	27.8 ± 0.11† (10)	0.35 ± 0.04* (10)	3.88 ± 0.30 (10)
11°C, full ration	17.9 ± 1.24 (10)	27.5 ± 0.17† (8)	0.50 ± 0.06 (9)	4.34 ± 0.22 (10)
15°C, reduced ration	20.6 ± 0.95 (10)	29.4 ± 0.28 (10)	0.41 ± 0.07 (9)	4.77 ± 0.25 (7)
15°C, full ration	18.4 ± 0.37 (10)	28.4 ± 0.33† (9)	0.48 ± 0.09 (8)	4.96 ± 0.38 (10)
19°C, reduced ration	18.9 ± 1.29 (10)	29.9 ± 0.25 (10)	0.44 ± 0.02* (9)	4.11 ± 0.28 (10)
19°C, full ration	19.7 ± 0.96 (10)	29.6 ± 0.33† (10)	0.60 ± 0.05 (10)	4.79 ± 0.31 (9)

Table 2. Summary of juvenile steelhead thermal preferences, thermal tolerances, metabolic rates and swimming performance. Significant differences among ration levels at the same temperature are denoted by (*). Significant differences among temperatures at the same ration level are denoted by (†). Sample sizes are shown in parentheses.

Discussion

SGRs were temperature-dependent, as expected, but were independent of ration level. At rations lower than the 79 - 88% used in this study, we would expect to see significant decreases in growth rate as less energy would be available for growth. Our results show that juvenile steelhead fed *ad lib*. rations are converting food into usable energy as efficiently as those fed on reduced rations. The loss of efficiency may be due to a re-partitioning of energy to other terms in the energy budget equation:

$$C = \left(M_r + M_a + SDA\right) + \left(F + U\right) + \left(G_s + G_r\right)$$ (Adams and Breck, 1990)

such as specific dynamic action (*SDA*), activity (M_a) or waste losses (*F, U*). Specific consumption rates were also temperature-dependent, indicating that steelhead increase their consumption rates to supply energy necessary for the temperature-related increases in growth rates.

With the exception of the 15°C steelhead, reduced ration treatments had lower mass-dependent oxygen consumption rates than full-ration treatments. This difference can probably be explained by the reduced SDA of the reduced-ration

treatments (Alsop and Wood, 1997). Surprisingly, we observed no temperature effect, suggesting that CA steelhead avoid costly increases in MO_2 over the 11 - 19°C range (Q_{10}-reduced: 1.33, Q_{10}-full: 1.26), which, theoretically, can lead to greater energy availability for growth and activity.

Although the differences were not statistically significant, full-ration steelhead tended to swim faster than reduced-ration fish at all temperatures. This trend suggests that full-ration steelhead are capable of mobilizing more energy reserves than reduced-ration fish and thus may be significantly faster than fish fed reduced rations lower than 80%.

Juvenile CA steelhead CTMs showed a significant thermal acclimation effect while ration had no effect. The significance of this finding is that CTMs are ration-independent, at least over the range tested. All juvenile steelhead preferred temperatures in the 17.9 - 20.6°C range, irrespective of acclimation temperature or ration level. This preferendum approximates the optimum temperature for growth and food consumption, in line with similar relationships shown by other species (Jobling, 1981).

Acknowledgments

The research on which this extended abstract is based was financed in part by the University of California Water Resources Center, Project UCAL-WRC-W-885 and by the Bob Bittner Memorial Scholarship Fund. Contents of this publication do not necessarily reflect the views and policies of the U.S. Department of the Interior, nor does mention of trade names or commercial products constitute their endorsement or recommendation for use by the U.S. Government. We thank T. Mussen, C. Peregrin and members of the UCD fish physiology lab for technical assistance and P. Lutes and B. Bentley for facilities support.

References

Adams, S. M. and Breck, J. E. (1990). Bioenergetics. In, Methods for fish biology (Schreck, C. B. and Moyle, P. B., eds.), pp. 389 - 415. Bethesda, Maryland: American Fisheries Society.

Alsop, D. H. and Wood, C. M. (1997). The interactive effects of feeding and exercise on oxygen consumption, swimming performance and protein usage in juvenile rainbow trout (*Oncorhynchus mykiss*). Journal of Experimental Biology 200: 2337 - 2346.

Jobling, M. (1981). Temperature tolerance and the final preferendum: rapid methods for the assessment of optimum growth temperatures. Journal of Fish Biology 19: 439 - 455.

TEMPERATURE AND GROWTH PERFORMANCE OF JUVENILE COMMON WOLFFISH (*ANARHICHAS LUPUS* L.)

Ian McCarthy
Fish Biology Group, University Field Station,
Rowardennan, Glasgow, G63 0AW, UK.
Tel. +44 (0)141 870271,
Fax +44 (0)141 870389
email idmc1m@udcf.gla.ac.uk

Erlend Moksness
Institute of Marine Research,
Flødevigen Marine Research Station, 4817 His, Norway.

Dimitri Pavlov
Chair of Ichthyology, Department of Biology,
Moscow State University, 119899 Moscow, Russia.

Introduction

The common wolffish, *Anarhichas lupus* L., is a benthic marine fish that has been identified as a possible candidate species for coldwater aquaculture in Northern Europe and Atlantic Canada (reviewed in McCarthy *et al.*, 1998a). In the past ten years, significant advances have been made in our ability to control the life history of the common wolffish in captivity and investigating the culture conditions that promote optimal development and growth (reviewed in Moksness & Pavlov, 1996). Although some progress has been made in determining the abiotic preferences of common wolffish under culture conditions and the effect of diet quality on growth (reviewed in Moksness & Pavlov, 1996), our knowledge of the optimum abiotic/nutritional combinations required to maximise growth during the various phases of the wolffish life-cycle (larvae, juvenile and adult) is still incomplete. Water temperature has been identified as the major abiotic factor affecting the growth and physiological performance of fish (Brett, 1979) and a knowledge of the temperature tolerance and optimum temperature for growth is essential in the development of wolffish aquaculture.

In this study, the optimum water temperatures for growth, food conversion efficiency and protein synthesis retention efficiency of juvenile common wolffish were determined.

Methods

In this study, duplicate groups of juvenile common wolffish (initial weight 26 g) were reared in 1 m tanks (water volume = 270 litres) at 5, 8, 11 and 14°C for 98 days and fed to satiation 2-6 times a day. Feeding frequency was dependent on feeding motivation at the 4 water temperatures. At the end of the experiment, fractional rates of protein synthesis and protein growth were measured in selected fish using a single flooding dose injection of L-(2,6)^{3}H-phenylalanine (reviewed in Houlihan *et al.* 1995). A complete description of the experimental design, information on the parameters measured or calculated and details of statistical analysis are provided in McCarthy *et al.* (1998a and b). In this extended abstract, only a summary of the main results obtained and the conclusions drawn from this study are presented. For a complete description of the statistical analysis underlying these conclusions, please refer to the original primary papers (McCarthy *et al.* 1998a and b) or contact the first author.

1) Consumption rates (expressed as % body weight.day^{-1}) increased with increasing water temperature between 5 and 11°C (Figure 1A).

2) Whole-body specific growth rates (expressed as % body weight.day^{-1}) increased between 5 and 11°C, were highest at 11°C and declined at 14°C (Figure 1B).

3) Growth efficiency (g weight gain per g food eaten) increased between 5 and 8°C, was highest between 8 and 11°C and declined at 14°C (Figure 1C).

4) Fractional rates of white muscle and whole-body protein synthesis (%.day^{-1}, expressed as a percentage of the final protein content of the white muscle or whole-body) increased in a linear fashion with increasing water temperature (Figure 1D).

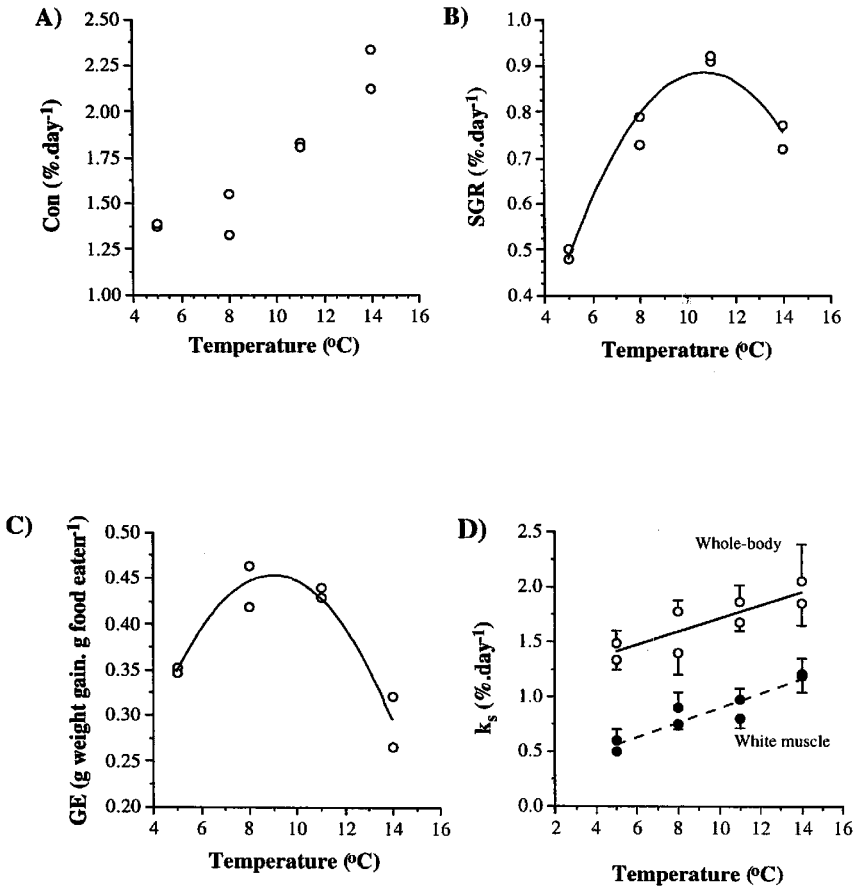
5) Whole-body and white muscle fractional protein growth rates (%.day^{-1}, expressed as a percentage of the final protein content of the white muscle or whole-body) increased between 5 and 11°C, were highest at 11°C and declined at 14°C (Figures 1E and F).

Results and Discussion

The results indicated that the optimum water temperatures for growth (T_{optG}) and growth efficiency (T_{optGE}) were 10-11 and 9-10°C respectively. Table 1 provides a comparison of the whole-body and white muscle fractional protein growth rates and protein synthesis retention efficiencies (k_g/k_s, protein growth divided by protein synthesis expressed as a percentage) for juvenile common wolffish at 9-11°C (i.e. the range covering their T_{optG} and T_{optGE} values) with values obtained for salmonid fish (the dominant culture species in coldwater aquaculture).

The growth rates in this study are lower than most of those recorded for salmonid fish at 10-12°C (Table 1). However, it is already possible to grow wolffish to a market size of 2 kg in two years (Moksness and Pavlov, 1996) and increased growth rates are anticipated as the optimal dietary formulation and pellet design for juvenile common wolffish become known and rearing conditions are optimised (McCarthy *et al.* 1998a).

In contrast, whole-body and white muscle k_g/k_s values were higher compared to those of salmonid fish (Table 1). This increased growth efficiency may be due to the reduced swimming activity and levels of aggression seen in common wolffish (McCarthy *et al.* 1998b). The growth performance results of this study further highlight the potential of wolffish as an alternative species for coldwater aquaculture.


A)
Con (%.day-1)
Temperature (°C)
B)
SGR (%.day-1)
Temperature (°C)
C)
GE (g weight gain. g food eaten-1)
Temperature (°C)
D)
Whole-body
White muscle
ks (%.day-1)
Temperature (°C)

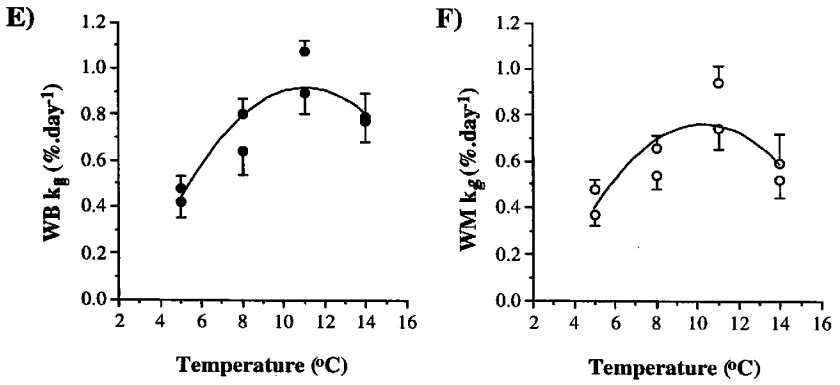


Figure 1. The relations between water temperature and:

(A) consumption rates (Con, %.day^{-1}),
(B) whole-body specific growth rates (SGR, %.day^{-1}),
(C) growth efficiency (GE, g weight gain. g food eaten^{-1}),
(D) whole-body and white muscle fractional rates of protein synthesis (k_s, %.day^{-1}),
(E) whole-body fractional rates of protein growth (WB k_s, %.day^{-1}) and
(F) white muscle fractional rates of protein growth (WM k_s, %.day^{-1}) of juvenile common wolffish (*Anarhichas lupus* L.).

Table 1. A comparison between the fractional protein growth rate (k_g, $\%.d^{-1}$) and protein synthesis retention efficiency (k_g/k_s , %) in the white muscle (WM) and whole-body (WB) of juvenile common wolffish and salmonid fish (original references cited in McCarthy *et al.* 1998b). The body weight (Wt, g) and temperature (T, °C) for each study are also presented.

Species	**Wt**	**T °C**	**WM/WB**	**k_g**	**k_g/k_s**
Common	65	9-11	WB	0.7	51
Wolffish			WM	0.9	92
Rainbow trout	65	10	WB	0.4	35
-			WM	1.0	67
-	65	12	WM	0.4	73
-	75	11	WB	2.0	45
-	108	10	WB	0.9	30
-			WM	1.3	52
Atlantic salmon	37	12	WB	1.6	53

Acknowledgements

This work was carried out with financial support from the Norwegian Research Council and the Carnegie Trust for the Universities of Scotland.

References

Brett (1979). Environmental factors and growth. In Fish Physiology Vol VIII Bioenergetics and Growth (eds Hoar, Randall & Brett). pp 599-675. Academic Press: New York.

Houlihan, Carter & McCarthy (1995). Protein synthesis in fish. In *Biochemistry and Molecular Biology of Fishes Vol. 4* (eds Hochachka & Mommsen). pp 191-200. Elsevier: Amsterdam.

McCarthy, Moksness & Pavlov (1998a). The effects of temperature on growth rate and growth efficiency of juvenile common wolffish. Aquaculture International 8, in press.

McCarthy, Moksness, Pavlov & Houlihan (1998b) Effects of water temperature on protein synthesis and protein growth in juvenile common wolffish (*Anarhichas lupus* L.). Canadian Journal of Fisheries and Aquatic Sciences, under review.

Moksness & Pavlov (1996). Management by lifecycle of wolfish, *Anarhichas lupus* L., a new species for coldwater aquaculture: a technical paper. Aquaculture Research, 27, 865-883.

OPTIMIZATION OF DOCOSAHEXAENOIC ACID (DHA, 22:6N-3) ENRICHMENT IN *ARTEMIA* NAUPLII THROUGH DIETARY AND ENVIRONMENTAL MANIPULATION

Angela Gubernatis
Center of Marine Biotechnology, University of Maryland
701 East Pratt St. Baltimore, MD. 21202

Moti Harel
Center of Marine Biotechnology, University of Maryland
701 East Pratt St. Baltimore, MD. 21202

Allen C. Place
Center of Marine Biotechnology, University of Maryland
701 East Pratt St. Baltimore, MD. 21202

EXTENDED ABSTRACT ONLY DO NOT CITE

Introduction

Standard enrichment procedures of live feed (*Artemia* nauplii and Rotifers) does not provide a sufficient level of DHA and the proper DHA to eicosapentaenoic acid (EPA, 20:5n-3) ratio required by marine and cold water fish larvae for optimal growth and survival (reviewed by (Watanabe 1993). Absorption of dietary DHA depends on the capacity of the live feed organism to digest and assimilate the emulsified enrichment lipids. This capacity may be affected by a variety of environmental and nutritional factors.

In the present investigation, with *Artemia* nauplii, the DHA incorporation rate as a function of the feeding ration, composition of enrichment lipids, enrichment period at different temperatures, and in relation to nauplii size was studied.

Methods

Enrichment diets were composed of varying proportions of DHA-rich phospholipids extracts (DHA-PL; 5, 10%) and DHA-rich sodium soaps (DHA-SS; 10, 20, or 40%) from *Crypthecodinium sp.* algae at a constant level of DHA (32±2% of total fatty acids). Diets were fed in three replicates to instar II-stage *Artemia* nauplii. Enrichments experiments were carried out over a 24 hour period at 24, 28, or 32°C, in 20ppt artificial sea water (200,000 nauplii/liter), provided with vigorous aeration and constant illumination by fluorescent light. Three equal portions of 0.3g enrichment lipids were mixed with fresh water, and fed to the Artemia nauplii at times 0, 8, and 16 hours.

Results and Discussion

Survival of 16 hours enriched *Artemia* nauplii was 74±7.8% and not significantly affected ($P>0.05$) by increasing quantities of dietary DHA-SS and DHA-PL (up to 30% and 40% respectively). Lipid content and composition of 16 hours enriched *Artemia* nauplii was equal in all dietary treatments (24.3±0.6%; $P>0.05$) and was independent of dietary composition (Fig. 2). DHA incorporation was directly correlated with *Artemia* growth rate and temperature. Maximum DHA incorporation rate was obtained at 20% dietary DHA-PL, or at dietary lipid composition of 10% DHA-PL, 10% DHA-SS and 80% DHA-rich triacylglycerol (DHA-TAG, Fig.1). However, a combination of 20% DHA-PL and 30% DHA-SS produced the highest ($P<0.05$) DHA:EPA ratio in 16 hour enriched *Artemia* nauplii. Furthermore, DHA was incorporated into *Artemia* at higher rates when delivered in PL or TAG form as compared with free ethyl-ester form (DHA-EE).

Results suggest that DHA accumulation in *Artemia* is growth dependent and occurred mostly within the first 16 hours post instar II-stage. Results also show that DHA absorption by *Artemia* nauplii can be improved significantly by dietary inclusion of polar lipids, mainly as DHA-PL and DHA-SS. This may be the result of the emulsifying properties of phospholipids and increasing proportions of preformed soft soluble sodium salts in the *Artemia* gut. In addition, although dietary PL were varied substantially, no significant change was observed in *Artemia* lipid class composition (Fig. 2) indicating that PL were digested into free fatty acids and deposited mainly as TAG. Similar observations were also reported in other studies (Tackaert et al., 1991; Rainuzzo et al., 1994).

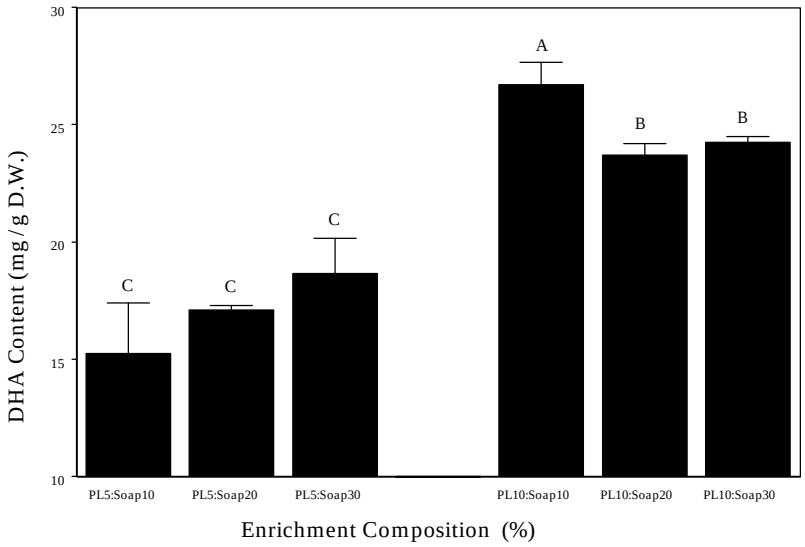


Fig. 1. The effect of dietary lipid composition on DHA accumulation in *Artemia* nauplii. *Artemia* were enriched for 16 h with a combination of 5 or 10% DHA-PL and 10, 20 and 30% DHA-SS. Bars represent the mean values with their standard errors (n=3). Bars having different letters are significantly different from each other ($P<0.05$).

References

Rainuzzo, J.R., Reitan, K.I., Jorgensen, L., and Olsen, Y. 1994. Lipid composition in turbot larvae fed live feed cultured by emulsion of different lipid classes. *Comp. Biochem. Physiol.* 107A: 699-710.

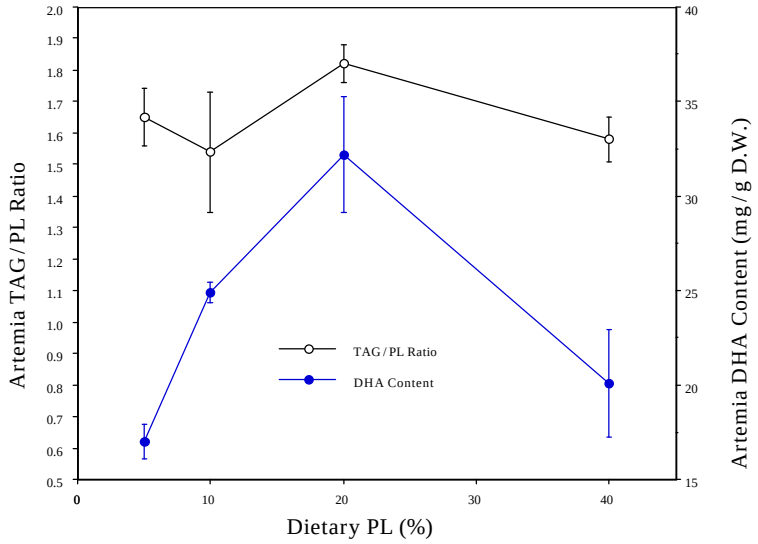


Fig. 2. The effect of varying DHA-PL:DHA-TAG proportions in dietary lipids on Artemia lipid composition and DHA content after 16 h enrichment. Each data point represents the mean values with their standard errors (n=3).

Tackaret, W., Camara, M.R., and Sorgeloos, P. 1991. The effect of dietary phosphatidylcholine in post larval penaeid shrimp. I Diet preparation. In: P. Lavens, P. Sorgeloos, E. Jaspers, and F. Ollevier (Editors), Larvi '91 - Fish and Crustacean Larviculture Symposium. Gent., Belgium. Europe. *Aquacult. Soc. Spec. Publ.* 15: 76-79

Watanabe, T. 1993. Importance of docosahexaenoic acid in marine larval fish. *J. World Aquacult. Soc.* 24: 152-161.

EFFECT OF SHORT-TERM FOOD DEPRIVATION AND ITS TEMPORAL PATTERN ON CONSUMPTION AND GROWTH PERFORMANCE OF THREE-SPINED STICKLEBACKS

M. Ali and R. J. Wootton
Institute of Biological Sciences, University of Wales,
Aberystwyth, Wales, SY23 3DA, U.K.
Tel.: 01970 622346; fax: 01970 622350;
e.mail: mua95@aber.ac.uk

Introduction

Studies on compensatory growth in fish have shown that a period of food deprivation is followed by a phase of hyperphagia (Miglavs and Jobling, 1989; Russell and Wootton, 1992; Koppe et al., 1993; Jobling, 1994; Paul et al., 1995, Hayward et al., 1997). However, the dynamics of the relationship between a period of deprivation and the subsequent hyperphagia are poorly understood. The aim of this study was to relate temporal patterns of food deprivation to the subsequent immediate hyperphagia. To define the rate of food consumption in the absence of deprivation, the study first determined the voluntary rate of consumption by the fish for whom food was always present.

Materials and Methods

Juvenile (0^+) three-spined sticklebacks were collected from Llyn Frongoch in Mid Wales. In the laboratory, fish were acclimatised to 14^0C and a photoperiod of 10L14D for two weeks. During both acclimatisation and experimental period, the fish were fed enchytraeid worms cultured in laboratory. Fish were left for 24h without feeding and then weighed (mg) and total length measured (mm). Fish were than assigned at random to 30 individual tanks, which were on filtered continuous flow systems. The daily food consumption was recorded for seven days when food was always present in the tanks. The fish were again left without feeding for 24h and then weighed and measured. The fish were then

divided randomly into six groups of 5 each and subjected to one day (1D), three days (3D) and six days (6D) food deprivation regime. For Group 1 the pattern of intervals was (6D-1D-3D), Group 2 (6D-3D-1D), Group 3 (3D-1D-6D), Group 4 (3D-6D-1D), Group 5 (1D-3D-6D), Group 6 (1D-3D-6D). At the end of 12 days experimental period, the fish were not fed for one day, killed and then weighed and measured. The fish were feeze dried and lipid content of individual fish was measured.

SPSS and MINITAB were used for all statistical analysis, which included ANOVA, ANCOVA and Repeated measure ANCOVA. All lengths and weights were log transformed and percentages were arc-sine transformed before analysis.

Results

The average daily food consumption expressed as % initial bw = 11.74% (95%CI= 0.98), (n= 30). The fish after short-term deprivation became hyperphagic and their average daily food consumption increased to 19.8% (95%CI= 1.96) at 1D interval, 22.3% (95%CI= 1.26) at 3D interval and 19.5% (95%CI= 1.67) at 6D interval. Repeated measures ANCOVA with initial length as a covariate ($F=20.03$, $df= 1, 23$, $p << 0.001$) showed that the overall mean weight of food consumed was not significantly different between the groups ($F= 1.79$, $df= 5, 23$, $p> 0.1$), (between treatment effect). However, there was a highly significant within subject interaction between food consumption and group ($F= 6.32$, $df= 10, 46$, $p< 0.001$)). The rate of consumption immediately after a given period of deprivation (1, 3 or 6D) depended on the previous pattern of deprivation the fish had experienced (Fig).

The temporal pattern of feeding had no significant effect on specific growth rate (G) ($F= 0.67$, $df= 5, 24$, $p>0.1$), lipid content ($F= 0.20$, $df= 5, 24$, $p>0.1$) and dry matter content ($F= 2.04$, $df= 5, 23$, $p>0.1$). Initial length used as a covariate had a significant effect ($F= 5.6$, $df= 1, 23$, $p = 0.027$) on dry matter content (Table 1).

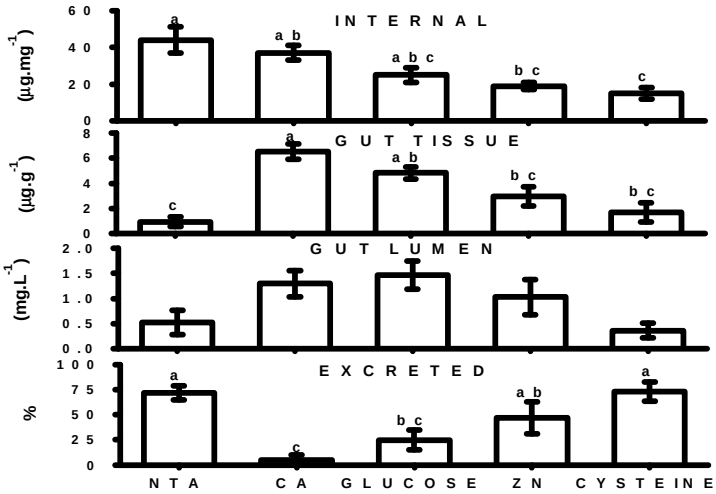


Figure 1. Effect of ligands on Cu (2.4 µmole) uptake after 48 h at 16°C.

Feeding pattern (95%CI)	Initial length mm (95%CI)	G d^{-1} (95%CI)	Lipid % (95%CI)	Dry matter % (95%CI)
Group 1 (N=5)	39 (1.80)	0.0084 (0.0031)	26.7 (1.79)	25.5 (0.71)
Group (N=5)	40 (2.92)	0.0116 (0.0056)	26.3 (0.45)	26.2 (0.62)
Group 3 (N=5)	38 (2.35)	0.0116 (0.0050)	26.2 (2.05)	24.7 (0.83)
Group 4 (N=5)	39 (3.46)	0.0086 (0.0025)	26.0 (1.41)	24.6 (0.67)
Group 5 (N=5)	37 (1.84)	0.0087 (0.0034)	25.7 (2.04)	24.7 (0.81)
Group 6 (N=5)	38 (2.58)	0.0080 (0.0029)	25.8 (1.29)	24.8 (1.35)

Table 1. Effect of temporal pattern of feeding on indices of growth in juvenile three-spined sticklebacks.

Discussion

The results suggested that the fish became hyperphagic after interrupted feeding. A previous experiment had shown that the fish fed at regular or random intervals showed hyperphagia, consuming as much as 21% of their body weight when food become available to compensate for days deprived of food. For the temporal pattern of feeding used, the fish were able to adjust their food intake and maintain their growth performance (Ali and Wootton, unpublished). The present experiment showed that the previous pattern of food deprivation was a factor in determining the degree of hyperpagia shown. However, the rate of consumption averaged over different temporal patterns of deprivation did not differ significantly. One interpretation of this is that fish adjust their food intake to maintain their growth performance.

Acknowledgements

M Ali is supported by studentship from Government of Pakistan.

References

Hayward, R. S., D. B. Noltie, and N. Wang. 1997. Use of compensatory growth to double hybrid sunfish growth rates. Transactions of American Fisheries Society 126: 316-322.

Jobling, M. 1994. Fish energetics. Chapman and Hall, London.

Koppe, W., J. Pockrandt, K. H. Meyer-Burgdorff, and K. D. Gunther. 1993. Effects of realimentation after a period of restricted feeding on food intake, growth and body composition in *Piaractus brachypomus* (Cuvier 1818), a South American characoid fish. Pages 263-268 *in* T. Braunbeck, W. Hanke, and H. Segner, editors. Fish ecotoxicology and ecophysiology. Proceedings of an International Symposium, Heidelberg, September 1991. VCH, New York.

Miglavs, I., and M. Jobling. 1989. Effects of feeding regime on food consumption, growth rates and nucleic acids in juvenile Arctic charr, *Salvelinus alpinus*, with particular respect to compensatory growth. Journal of Fish Biology 34: 947-957.

Paul, A. J., J. M. Paul, and R. L. Smith. 1995. Compensatory growth in Alaska yellowfin sole, *Pleuronectes asper*, following food deprivation. Journal of Fish Biology 46: 442-448.

Russell, N. R., and R. J. Wootton. 1992. Appetite and growth in the Europian minnow, *Phoxinus phoxinus* (Cyprinidae), following short periods of food restriction. Journal of Fish Biology 34: 277-285.

EMBRYONIC MUSCLE DEVELOPMENT

IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

USING SCANNING ELECTRON MICROSCOPY

AND IMMUNOHISTOLOGY

Julien M. Bobe
INRA, Laboratoire de physiologie des poissons
Campus de Beaulieu
35042 RENNES Cedex FRANCE
Phone: (219) 631 4571/Fax: (219) 631 7413
Julien.Bobe.1@nd.edu

Sylvie Andre and Benoit Fauconneau
INRA, Laboratoire de physiologie des poissons

Summary

Early muscle development was studied in rainbow trout using scanning electron microscopy and immunohistology. Somite size and shape were characterized, starting at stage 16 (according to Vernier, 1969). Myogenesis initiates in the deep somite, near the notochord at the 20 to 55 somites stage. Fast myosin is expressed first at stage 20 in the deep somite and slow myosin is expressed at stage 24 (eyed stage), in the somite periphery. At hatching (stage 30), the entire somite is composed of white muscular fibers exhibiting a high degree of maturity.

Introduction

The aim of this study was to describe morphological and functional aspects of muscle development during the embryonic stages of rainbow trout. Myogenesis was analysed through the expression of myosin inside the somite.

Materials and Methods

Eggs were incubated either at 4°C ± 0.5 or 12°C ± 0.5. Samples were collected every 50 degree.days at the two temperatures from fertilization up to the eyed stage. Following this, samples were taken at the same developmental stages. Eggs were dissected and the embryos removed from the chorion and observed directly by light microscopy. Other embryos were treated for SEM or immunohistology. All developmental stages were identified according to stages reported by Vernier (1969).

Scanning Electron Microscopy (SEM): After fixation in Karnovsky fixative (4% paraformaldehyde, 5% glutaraldehyde, 0.08 M sodium cacodylate) and 0.1% osmium tetroxide, samples were dehydrated in ethanol, dried and observed. The skin was removed and fractures were made in the embryo to observe somites and the characteristics of contractile elements.

Immunohistology : Samples were fixed in glycine-ethanol buffer and embedded in paraffin. Thin transverse sections (5 µm) of the embryos were made and put on TESPA (Sigma A-3648) treated glass. Primary antibodies directed against fast and slow myosin were applied on desembedded sections. A secondary antibody, labelled with FITC (Fluoresceine iso thiocianate) and directed against the primary antibody was used in the immunofluorescence study to localize myosin. Sections were observed using a light fluorescence microscope.

Results

Observations made on muscle and general embryonic development using either light or scanning microscopy are presented in Table 1.

Myosin expression was first observed at stage 20 using a fast MHC (myosin heavy chain) monoclonal antibody ($S_4$8E6). The expression was limited to the very deep part of the somite near the notochord at stage 20 and 22. At stages 24 and 26, the area of myosin expression expanded laterally with a gradient of intensity from the deep part of the somite to the periphery in agreement with the gradient in muscle differentiation described in zebrafish (Waterman, 1969;

TABLE 1: Description of muscle development at different embryonic stage(degree.d : degree.days).

Stage Number (Vernier 1969)	Stage Name	degree.d at 12 C	Muscle development	General embryonic development
10-11	Blastula	48		
16-18		96	20 to 30 somites Pillow to oval shape	
20-22		144	55 to 65 somites Chevron shape Muscle contractions	Heart beating 2 to 3 gill vents Pectoral fins Caudal Bud detached Pronephros
24	Eyed Stage	196	Myotubes within the deeper part of somites	Optical vesicle pigmented 4 gill vents Complete embryonic fin Curvature of caudal part Opening of mouth Opening of Kidney
26		252	Myofibrils Cross-striation	Anal and dorsal fins Lower maxillary Olfactory cavity
30	Hatching	336	Fibres Gradient of size	Pelvic fins Olfactory cavity doubled with ciliated cells Gills arch

Devoto *et al*., 1996). Myosin expression was observed in the whole somite at hatching (stage 30). The same results were observed with a fast MHC monoclonal antibody $S_4$10H9 except that no expression was observed until stage 22.

No myosin expression was observed until stage 24 using a BA-D5 monoclonal slow MHC antibody. At stage 24 (eyed stage) slow myosin expression was localised at the periphery of the somite in elongated monolayer cells exhibiting a V shape at the horizontal septum. The expression of slow myosin expanded dorsally and ventrally at stage 30 (hatching). Such differential expression of myosin isoforms has never been described in salmonids but it was suspected by early TEM analysis (Nag and Nursall, 1972). This sequential pattern is different to that observed in zebrafish where slow myosin expression starts in the deep part of the somite (Devoto et al. 1996).

Conclusions

Specific stages for muscle differentiation were identified. Description of muscle development during embryogenesis was done for the first time in trout. These results demonstrated a sequential expression of fast and slow myosin with late expression of slow myosin in superficial cells of the somites. This pattern is different from what was observed in other models such as zebrafish.

Acknowledgments

Supported by EC grant FAIR CT PL 96-1941

References

Devoto, H. S., Melancon, E., Eisen, J. and Westerfield, M. 1996. Identification of separate slow and fast muscle precursor cells in vivo, prior to somite formation. Development. 122: 3371-3380.

Nag A.C., Nursall, J.R. 1972. Histogenesis of white and red muscle fibres of trunk muscles of a fish Salmo gairdneri. Cytobios, 6: 227-246.

Vernier, J. M. 1969. Table chronologique du dÈveloppement embryonnaire de la truite arc-en-ciel, Salmo gairdneri Rich. 1836. Ann. Embryol. MorphogÈnËse. 4 (2): 495-520.

Waterman, R. E. 1969. Development of the lateral musculature in the teleost Brachydanio rerio: a fine-stuctural study. Am. J. Anat. 125: 457-494.

INFLUENCE OF TEMPERATURE ON LARVAL WALLEYE DEVELOPMENT, BEHAVIOR, AND VIABILITY IN TANK CULTURE

Phillip W. Rieger
Mead & Hunt, Inc., 6501 Watts Rd., Madison, WI, USA
Tel: +1-608-273-6380 E-mail: wateres@meadhunt.com

Robert C. Summerfelt
Department of Animal Ecology, Iowa State University, Ames, IA, USA
Tel: +1-515-294-6107 E-mail: rsummerf@iastate.edu

Abstract

Larval walleye behavior, performance, and development at 140C, 17.50C, and 200C are described. Swimming speed, cannibalism, mortality, gas bladder inflation (GBI), and growth were determined from daily observations and from examination of larvae surviving to the accumulation of 300 TUs (cumulative daily mean temperature, 0C). Growth and viability (the product of survival and GBI) were significantly greater at higher temperatures. Swimming speed, cannibalism, and mortalities had different temporal patterns at each of the three temperatures corresponding to accelerated development at higher temperatures. In tank culture, higher rearing temperature seems to improve GBI and first feeding success through enhanced vigor (measured as swimming speed) during the early larval phase. This enhanced vigor seems to also influence the timing and frequency of cannibalism and related mortalities. Our study provides strong evidence that the influence of temperature on critical developmental events can be used to increase viability of some fishes.

Introduction

Walleye (*Stizostedion vitreum*) popularity has increased the need for application of biotechnology to enhance sports and commercial walleye production. In 1983 and 1984, one billion walleye larvae were stocked throughout North America (Conover 1986). Mortality of larvae stocked in natural waters is, however, reported to exceed 90% (Loadman et al. 1986). Intensive culture on

artificial diets could provide an alternative to pond culture with minnows for raising walleye to lengths greater than 150 mm for stocking with improved survival. Attempts to intensively culture walleye larvae have, however, had limited success. A review of research revealed that survival of walleye through the larval period in tank culture rarely exceeded 50% and more often is less than 15% (Moore et al. 1984). Methods to improve walleye viability through the larval stage have been studied by many researchers in recent years.

Low survival of walleye in larviculture is known to occur primarily during a 2-week period that begins, not immediately after hatching, but at about one week posthatch when the larvae make the transition from yolk sac to exogenous feeding (Noble 1972). Li and Ayles (1981) termed this mortality event a "critical period." It has been hypothesized that an energy/nutrient deficit or poor adaptation to first feeding is causing starvation (Kindschi and MacConnel 1989; Loadman et al. 1989; Nagel 1991). Cannibalism is considered a contributory factor (Doepke 1970; Beyerle 1975; Cuff 1977; Li and Mathias 1982; Loadman et al. 1989), and may also be related to nutrient deficiencies (Loadman et al. 1986; Li and Mathias 1982). Initial gas bladder inflation (GBI) also occurs during this critical period. It is reported that poor gas bladder inflation (GBI) is common in tank culture and contributes to poor viability in walleye larviculture (Nickum 1987; Colesante et al. 1986; Barrows et al. 1988; Summerfelt 1991). If first inflation does not occur by the end of the critical period, the gas bladder degenerates and the fish is without hydrostatic control. Poor GBI among walleye larvae may also inhibit feeding success and contribute to cannibalism. As with feeding and cannibalism, GBI may be related to energy expenditures, nutrient reserves, and developmental rate during the first two weeks posthatch.

A review of larval walleye research reveals that a range of water temperatures from 14.50C to 250C has been used to rear walleye larvae in experimental studies (Table 1). Some investigators have noted that in the absence of controlled, replicated studies that indicate otherwise, it seemed more reasonable to rear walleye larvae at temperatures of 100C to 150C + the range occuring during larval walleye development in the natural environment. More recently, some studies have indicated that higher temperatures may result in better survival and feeding ability.

We initiated controlled studies to test whether temperature influenced a variety of developmental and behavioral characteristics that might affect viability of larval walleye in tank culture. We compared GBI success, growth, survival,

cannibalism, swimming speeds, and viability. Findings describing the interrelationships of temperature and these parameters during the critical period were of special interest.

Table 1. Temperature regimes used or recommended by various investigators for intensive larviculture of walleye.

REFERENCE	TEMPERATURE
Li & Mathias (1972)	20°C
Smith & Koenst (1975)	21°C
Hokanson (1977)	Increasing by 1°C per day
Nickum (1978)	Less than 20°C
McElman & Balon (1979)	15°C
Colesante et al. (1986)	11.5°C - 18.3°C
Summerfelt et al. (1991)	14.7°C - 19.0°C
Moore et al. (1994)	14.5°C - 25.0°C

Methods and Materials

Walleye larvae were reared in 3-liter glass aquaria with gravity-fed recirculating water supplied at one interchange per hour. Sumps for separate circulating systems included biofilters for water quality, and chillers to maintain constant temperatures. Three water temperatures were the treatments with replicate aquaria as experimental units: 14°C (3 aquaria); 17.5°C (4 aquaria); and, 20°C (3 aquaria). Larval densities in the aquaria were highly variable throughout the study because of different mortality rates occurring within the rearing units. Other environmental conditions (e.g., lighting, tank construction, feeding rate, turbidity, and flow rates) were identical for all rearing units in the study.

Daily mortality and cannibalism were determined by counting individual dead and cannibalized larvae each day when the aquaria were cleaned. Percent GBI and growth were determined by examination of all larvae surviving to termination of the rearing periods. Each experimental unit was reared to an end point accumulation of 300 TUs: 20°C test ended at 15d; 17.5°C at 17d; and, 14°C at 21d.

Videography was used to provide samples of larval behavior each day during the study for each temperature regime. Each day, 8-min long overhead video recordings were made of each of the aquaria. We used a Sony CCD color video camera with a Sony Beta VCR, which provided high-resolution recording with various replay options to enhance observations and analyses of recorded information. All larvae in the tanks were instantaneously visible in the video recordings. These observations were used to estimate mean swimming speed and describe cannibalistic behavior. Mean swimming speeds for each experimental unit were estimated by tracing, with color markers on a Mylar overlay, the paths of representative larvae shown on the video replay at ¼ real speed. The distance of the path and time of duration were used to calculate larval swimming speeds.

Comparisons of performance for the three temperatures were made by analysis of covariance (ANCOVA) for fish length, total cannibalism, GBI, and viability determined at the end of the test period. ANCOVA was used, because of the variable and changing larval densities, to consider the significance of the influence of density on other comparisons. In the absence of significant interaction ($P<0.05$), the influence of density interaction was removed for temperature comparisons. If density interaction was significant, the significance of temperature influence would be determined without the interaction removed. All comparisons were determined significant at the 95% level (i.e., $P<0.05$). Daily % cannibalism, % mortality, and swimming speed means were also graphed to display temporal patterns for these parameters at each of the three temperatures.

Results

Interaction by density as a covariate did not significantly influence comparisons among temperature treatments for any performance comparison (Table 2, *P*-value interaction). Temperature differences without interaction were therefore used for significance of differences among performance parameters for the three temperatures (Table 2, *P*-value wo interaction) .

Table 2. Performance of larval walleye reared at three temperatures to 300 TUs where n (aquaria) are experimental units. Values are means ± standard error. Analyses are derived from ANCOVA.

Temp (0C)	n	Density (fish/L)	Length (mm)	Cannibalism (%)	GBI (%)	Survival (%)	Viability (%)
20.0	3	30	17.0±0.43	22.1±2.64	98.0±2.00	22.0±3.46	21.6±3.49
17.5	4	53	12.2±0.19	21.1±1.73	85.9±8.54	22.0±1.08	19.0±2.34
14.0	3	21	10.5±0.07	7.0±1.63	74.7±8.51	15.0±4.04	10.7±2.14
P-value Temp w Int			0.0001	0.0021	0.1877	0.2113	0.0652
P-value Interaction			0.3566	0.8625	0.8181	0.9196	0.8934
P-value Temp wo Int			0.0001	0.0120	0.2403	0.0699	0.0124

Survival and GBI seemed greater at higher temperatures, but the differences were not significantly influence by temperature: survival ranged from 15% to 22% (*P*=0.0699), and GBI ranged from 75% to 98%, (*P*=0.2403). Viability, the product of these parameters, was however, significantly influenced by temperature (*P*=0.0124), being approximately twice as high at both higher temperatures than at 140C. Temperature effects on growth were also significant; mean lengths were 14 mm at 200C and 10.5 mm at 140C (*P*=0.0001) at the end of 300 TUs. Performance values of the mid temperature (17.50C) were always between the higher and lower temperatures.

Temporal analyses of some parameters, along with swimming speed as a measure of activity level or larval vigor, further describe the differences and relationships among these parameters for each of the temperature units.

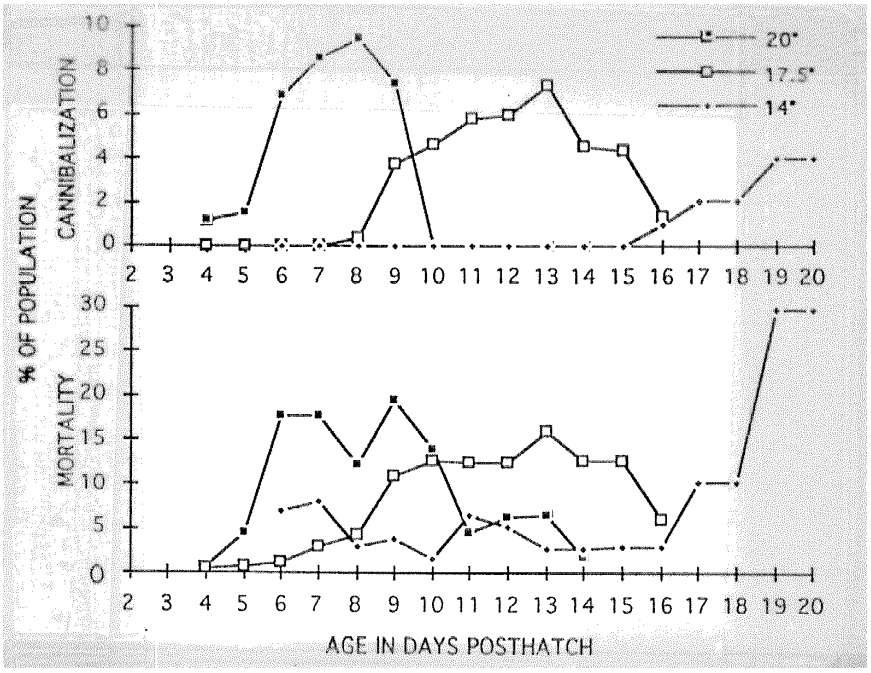


Figure 1. Temporal comparison of cannibalism and mortality for walleye larvae at three temperatures (0C)

The timing, duration, and intensity of cannibalism and mortality were distinctly episodic and noticeably different among the three temperatures (Figure 1). The temporal patterns exhibited by each temperature regime correspond to the different developmental rates that would be expected from the temperature range.

The ANCOVA of swimming speeds also show significant effects of temperature on activity levels. The range in mean swimming speed among the three temperatures was from 1.17 to 3.77 cm/sec. Interestingly, this high and low mean speed were both measured from the 200C rearing temperature (Figure 2).

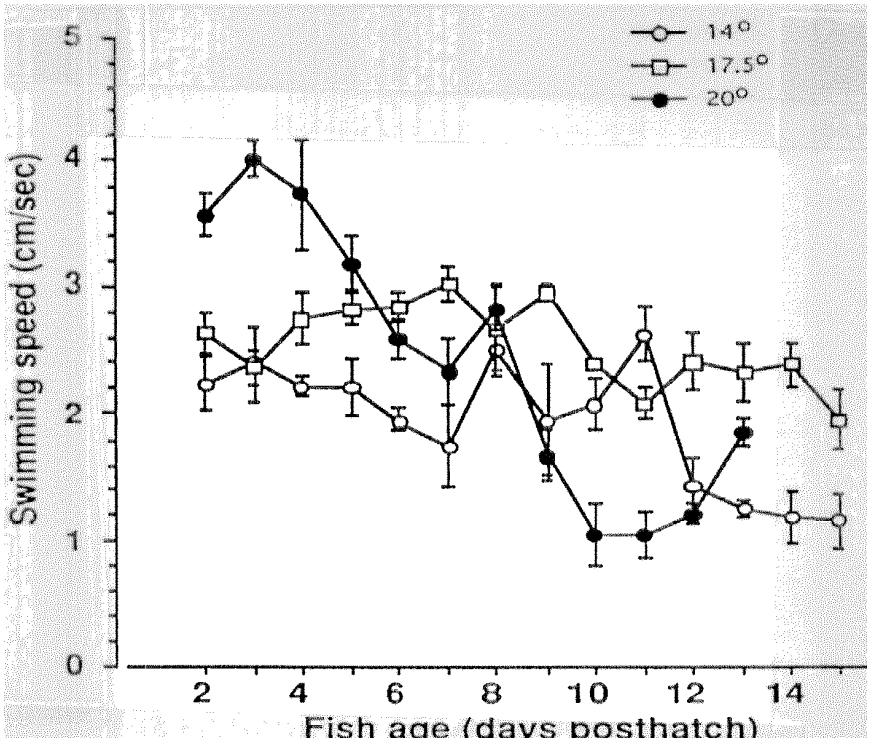


Figure 2. Temporal comparison of mean swimming speeds for walleye larvae at 3 temperatures (0C).

Although generally , in our study, younger fish and fish reared at higher temperatures had faster swimming speeds , there was a pronounced pattern for daily swimming speeds for larvae reared at 200C that was not apparent for lower temperatures. Larvae reared at 200C exhibited both the highest and lowest mean speeds + 3.77 cm/sec to 1.17 cm/sec. This pattern at 200C produced speeds higher early, then lower toward the end of the study. Swimming speeds for the two lower temperatures did not exhibit such a striking trend , but decreased gradually throughout the study , and were generally not significantly different from each other. The temporal descriptions for mortality,

cannibalism, and swimming speed all seem to follow a similar pattern, which varies in intensity and duration with temperature. An initial period of high activity, which begins soon after hatch, is followed by high cannibalism and mortality levels and decreasing activity levels. This episodic interrelationship among activity level, cannibalism, mortality, and temperature was most pronounced at 200C.

Discussion

Our study shows that temperature can significantly influence behavior, performance, and development of walleye larvae in tank culture. Higher temperatures increase the initial activity level of larvae (as measured by swimming speed), which seems to improve GBI and first feeding success. Although GBI was somewhat greater at higher temperatures, the differences were not statistically significant until combined with mortality, where significantly improved viability was observed. Larvae reared at higher temperatures were significantly larger at 300 TUs. Johnston and Mathias (1994) reported a higher attack rate of walleye larvae on zooplankton at temperatures ranging from 150C to 220C. Our findings support theirs, and further demonstrate, unfortunately, that this higher attack rate seems to also result in higher cannibalism, and possibly, cannibalism-related mortalities.

There was very little difference in total cannibalism between the two higher temperatures, but the lower temperature showed significantly less total cannibalism than both higher temperatures. Differences in overall mortality were not significant. At the end of the study, however, the period of cannibalism and mortality did not seem to have ended in the 140C population at 300 TUs (in fact they were increasing), although the critical period was elapsed for the higher temperatures. It seems very likely that, had the study been conducted beyond 300 TUs, cannibalism and mortality would have significantly increased in the 140C treatment. It seems that behavior and development of walleye larvae is not directly proportional to TUs. As such, we cannot conclude that overall cannibalism or mortality is actually less at the lower temperature. From the data obtained in this study, viability of larvae reared at 200C was twice as high as for those reared at 140C ($P=0.0124$); with higher mortality and cannibalism that would likely have been observed were the study for 140C extended beyond 300 TUs, however, the differences in viability would be even greater.

Cannibalism, high mortality, and reducing activity levels occurred nearly simultaneously during a discrete period at each temperature regime. This would be the "critical period" first defined by Li and Ayles (1981). In our study, this period occurred from 5d to 10d at 200C; 9d to 16d at 17.50C; and, 16d to >20d at 140C. Higher temperatures accelerated development, with sooner initiation, higher rate, and shorter duration of cannibalism and mortality. It is notable that the trends in daily mortality followed the same pattern and time frame as cannibalism. Rieger (1995) showed strong evidence that most of the unidentified mortality found in larval walleye culture was caused by injury from cannibalistic attacks.

Better growth and better viability at higher temperatures + but still, survival rates far less than 50%. It seems the key to high viability of larval walleye in tank culture may be reduction in cannibalistic behavior. The problem is that cannibalistic activity may actually be part of developing feeding behavior. It seems that when the larvae become successful exogenous feeders, their activity level lowers and cannibalistic behavior soon disappears. We have shown that this period of high mortality is discrete and influenced by temperature, both in intensity and duration. It may, therefore, be possible to use temperature manipulation to shorten the duration of the critical period without increasing the intensity of cannibalism and concomitant mortality.

Of interest, therefore, are the differences in intensity and timing of the critical period at different temperatures. Figures 2 and 3 provide a temporal framework from which alterations in temperatures can be hypothesized to influence cannibalistic behavior. For example, before GBI and first feeding (i.e., prolarvae), higher temperatures might be used to promote greater activity (i.e., vigor) to improve GBI and initiation of feeding; when the period of high cannibalism and mortality begins, the temperature would be lowered to reduce the intensity of cannibalistic activity. The population has, however, developed GBI and feeding behavior. Even at reduced activity level, therefore, feeding and advanced swimming behavior, should continue resulting in greater viability of the larvae to the end of the larval period.

Acknowledgments

This research was supported by funding from an American Fishing Tackle Manufacturers Association Boehme Fellowship and the Iowa Agriculture and Home Economics Experiment Station. Videographic assistance was provided

by the Iowa State University Office of Biotechnology Image Analysis Facility. Laboratory space and miscellaneous supplies were provided by Iowa State University Department of Animal Ecology.

References

Barrows, F.T., W.A. Lellis, and J.G. Nickum. 1988. Intensive culture of larval walleyes with dry and formulated feed: note on swimbladder inflation. Progressive Fish-Culturist 50: 160-166.

Beyerle, G.G. 1975. Summary of attempts to raise walleye fry and fingerlings on artificial diets, with suggestions of needed research and procedures to be used in future tests. Progressive Fish-Culturist 37: 103-105.

Colesante, R.T., N.B. Youmans, and B. Ziolkowki. 1986. Intensive culture of walleye fry with live food and formulated diets. Progressive Fish-Culturist 48: 33-37.

Conover, M.C. 1986. Stocking cool-water species to meet management needs. Pages 31-39 *in* R.H. Stroud, ed. Fish Culture in Fisheries Management. Fish Culture Section and Fisheries Management Section of the American Fisheries Society, Bethesda, Maryland.

Cuff, W.R. 1977. Initiation and control of cannibalism in larval walleyes. Progressive Fish- Culturist 39: 29-32.

Doepke, P.A. 1970. An ecological study of the walleye, *Stizostedion vitreum*, and its early life history. Ph.D. dissertation, University of Wisconsin, Madison, WI.

Hokanson, K.E.F. 1977. Temperature requirements of some percids and adaptations to the seasonal temperature cycle. Journal of the Fisheries Research Board of Canada. 34: 1524-1550.

Johnston, T.A. and J.A. Mathias. 1994. The effects of temperature on feeding in zooplanktivorous walleye, *Stizostedion vitreum*, larvae. Environmental Biology of Fishes. 40: 189-198.

Kindschi, G.A., and E. MacConnell. Factors influencing early mortality of walleye fry reared intensively. Progressive Fish-Culturist 51: 220-225.

Li, S., and G.B. Ayles. 1981. Preliminary experiments of growth, survival, production and interspecific interactions of walleye (*Stizostedion*

vitreum) fingerlings in constructed earthen ponds in the Canadian prairies. Canadian Technical Report of Fisheries and Aquatic Sciences 1041.

Li, S., and J.A. Mathias. 1982. Causes of high mortality among cultured larval walleyes. Transactions American Fisheries Society 111: 710-721.

Loadman, N.L., G.E.E. Moodie, and J.A. Mathias. 1986. Significance of cannibalism in larval walleye (*Stizostedion vitreum*). Canadian Journal of Fisheries and Aquatic Sciences 43: 613-618.

Loadman, N.L., J.A. Mathias, and G.E.E. Moodie. 1989. Method for the intensive culture of walleye. Progressive Fish-Culturist 51: 1-9.

McElman, J.F., and E.K. Balon. 1979. Early ontogeny of walleye, *Stizostedion vitreum*, with steps of saltatory development. Environmental Biology of Fish. 4: 309-348.

Moore, A., M.A. Prange, R.C. Summerfelt, and R.P. Bushman. 1994. Evaluation of tank shape and a surface spray for intensive culture of larval walleyes fed formulated feed. Progressive Fish-Culturist 56: 100-110.

Nagle, T.O. 1991. Artificial propagation of walleye. Pages 259-261 *in* Proceedings of the North Central Aquaculture Conference, Michigan State University, East Lansing.

Nickum, J.G. 1978. Intensive culture of walleyes: The state of the art. American Fisheries Society Special Publication 11: 187-194.

Noble, R.L. 1972. Mortality rates of walleye fry in a bay of Oneida Lake, New York. Transactions of the American Fisheries Society 101: 720-723.

Rieger, P.W. 1995. Behavior of larval walleye. Ph.D. Dissertation. Iowa State University, Ames. 130 pp.

Smith, L.L., Jr., and M.M. Koenst. 1975. Temperature effects on eggs and fry of percoid fishes. U.S. Environmental Protection Agency, Ecological Research Series, EPA - 660/3-75-017.

Summerfelt, R.C. 1991. Non-inflation of the gas bladder of larval walleye (*Stizostedion vitreum*): Experimental evidence for alternative hypotheses of its etiology. Pages 290-293 *in* P. Larens, P. Sorgeloos, E. Jaspers, and F. Ollevier, eds. Larvi' 91 - Fish and Crustacean Larviculture Symposium. European Aquaculture Society, Special Publication No. 15, Gent, Belgium.

ANALYTICAL METHODS USED TO EVALUATE BIOCHEMICAL STOCK IDENTIFICATION IN SALMONID FRY

John L. Sternick
Mansfield University, Grant Science Center, Mansfield, PA 16933,
jsternic@mnsfld.edu

and William F. Krise,
United States Geological Survey, Research and Development Laboratory,
Rural Delivery #4, Box 63, Wellsboro, PA 16901;
krisecol@epix.net.

EXTENDED ABSTRACT ONLY DO NOT CITE

In this study, salmonid fry are exposed to molecular markers foreign to fish, which can be used as a detectable tag in a stock identification mark system. The mark is intended to be placed in salmonid fry and recovered in juveniles, or if possible, adults. To produce this antibody mark, fish are exposed to a protein antigen bath for 5 to 10 minutes, rinsed in clean water, and under some circumstances are exposed to a second antigen bath in order to provide a greater concentrated exposure .

We have conducted dosage rate and longevity tests of mark retention with the Atlantic salmon (*Salmo salar*) and lake trout (*Salvelinus namaycush*). There have been no treatment-related mortalities, and serum from juvenile fish can be collected without sacrificing juvenile fish. Protein marks currently in use include bovine serum albumin, avidin, and hapten/carrier molecules. Most development has occurred with the use of bovine serum albumin which appears to slowly clear the fish's immune system. After exposure, we measure the mark concentration using the ELISA method and the avidin/biotin complex which magnifies the signal. Serum is collected, diluted at a 1:1 ratio in PBS, and aliquoted into 96-well ELISA plates. After blocking the plate, Tween 20 is added to the wash buffer to reduce background interference. Avidin-biotin

complex with horseradish peroxidase is used to obtain colorimetric results. The avidin/biotin complex method can also be used to evaluate fluorescent-tagged marks by adding a second enzyme tag to the serum in the microplate wells. This second method using ELISA plates includes the use of fluorescent tags which can be distinguished from one another at their peak absorbance/emittance wavelengths. Fluorescence is measured in microplate wells using a fluorescent reader and does not require the use of an actual ELISA. However, an anti-antibody can be added to serum with fluorescent tag present, and the assay also run as an enzyme assay. These methods have employed the use of biotinylated antigens, a rinse, then an avidin bath with an avidin-fluorescent tag used to determine presence of fluorescence in serum.

We also use gel electrophoresis as an alternative or confirmatory method to identify the presence of serum proteins used as antigens. These gels show the presence or absence of proteins of the same molecular weight as the antigen in serum. Gels are developed using molecular weight markers and control serum to indicate molecular weights of serum proteins in previously immunized fish. Although these tests have been conducted with tagged markers requiring blood samples for analysis, our goal is to read the molecular mark directly in fish fins, or the skin surface which would eliminate the need for blood sampling.

These initial methods have provided an indication that we can successfully immunize salmonid fry before first feeding and detect the presence of small amounts of the antibody and its tag in serum as long as one year after immunization.

LONG-TERM DETECTION OF BOVINE SERUM ALBUMIN MARKER IN SALMONID FRY SERUM BY ELISA

John L. Sternick
Biology Department, Grant Science Center
Mansfield University
Mansfield, PA 16933

William F. Krise
United States Geological Survey
Research and Development Laboratory
Rural Route #4, Box 63
Wellsboro, PA 16901

Abstract

These studies were designed to determine whether or not molecular markers were detectable in blood and serum, and to find the lowest dose detectable by ELISA. In addition, we tested for *in vitro* binding of avidin to biotinylated bovine serum albumin (B-BSA) in fish serum and evaluated Atlantic salmon marked as fry with 3.1 Tg/ml B-BSA 14 months prior to sampling.

To establish an ELISA standard curve for B-BSA in blood and serum from several juvenile Atlantic salmon (*Salmo salar*), blood was collected and pooled. Blood and serum samples were aliquoted and spiked with various concentrations of B-BSA (6 Tg/ml to 90 Tg/ml) and ELISA performed. ELISA were also performed on B-BSA exposed fish. Levels of B-BSA were found in serum and compared to standard B-BSA curves.

Detection of B-BSA concentrations in blood were at least one-third the value to those found in serum when comparing fish exposed to the same initial B-BSA concentration. The lower level of B-BSA detection using the avidin-BSA-enzyme complex was between 3.75 and 1.88 Tg/ml. Nonspecific protein binding did not occur until serum was diluted in PBS at a rate of 1:10,000, well below the 1:1 dilutions used in assays. We were able to detect the presence of B-BSA in seven of eight Atlantic salmon 14 months after their exposure.

These results indicate that low levels of B-BSA in serum or blood could be detected by ELISA, and that the response is saturable.

Introduction

This study presents a method for marking larvae or fry of salmonids or other fishes, and provide a practical means of stock identification over an extensive time period. We employ non-toxic, slow-clearing, tagged proteins to tag batches or groups of small fish. The molecular marks, are read as the fish grows, in order to determine if the marks are readable in juvenile and adult fish. Also, it is our goal to use a non-lethal sampling procedure to retrieve the mark. This study will also show that biotinylated bovine serum albumin (B-BSA) can form a chemical complex with avidin in blood or serum of the fish and be detectable at low levels. The method of bath marking can provide advantages such as non-lethal sampling, a simple and quick method for exposure, and a simple procedure for distinguishing stock which will not require sophisticated equipment or expert training as other methods such as genetics evaluations (Nielsen 1992). This current method would be applicable for marking fry or larvae before they begin feeding, and would be a group mark rather than individual marks used for larger juveniles such as coded wire tags, brands or elastomer tags (Johnson 1995, Moffett et al. 1997, Dussault and Rodriguez 1997).

The purposes of this study were:

- to determine lower limits of detection of molecular marks using ELISA,
- to determine the extent and effects of non-specific protein binding on serum ELISA, and
- to refine ELISA methods by using Tween 20 in wash buffers, varying ELISA incubation times and serial dilution of serum to find minimum detectable levels of molecular marks.

The test results will determine molecular mark concentrations needed to optimize the sensitivity of the method.

Materials and Methods

Several *in vitro* ELISAs were performed respectively with Atlantic salmon (*Salmo salar*) blood, serum, and with phosphate buffered saline (PBS) as control. These media carried various concentrations of biotinylated bovine serum albumin (B-BSA), or avidin (Pierce, Rockford, IL). ELISA procedures are described according to the type of result anticipated for each. Some procedures common to all assays, blood collection and handling, ELISA procedures such as blocking or washing of microplates, will be abbreviated after their first description.

Determination of B-BSA coupled to avidin in serum or blood

Three concentrations of B-BSA (either 0.090 or 0.048 Tg/ml, 0.024, and 0.006 Tg/ml BSA) were added to whole blood or serum pooled from 12-20 juvenile Atlantic salmon and used in ELISA of B-BSA. Two to three mls of blood was collected from anesthetized (in methane tricaine sulfonate) 2 year old Atlantic salmon from caudal puncture in 3cc syringes, and pooled into 20 ml samples. Heparin, 250 units/ml, was added to whole blood to prevent clotting, and blood used for serum collection was refrigerated overnight and centrifuged for 10 min at 13,000 rpm to separate serum from blood cells. After separation, serum was drawn off and stored frozen until use. Blood collected for serum did not include the use of heparin.

In these assays, 25 Tg/ml avidin was added to blood or serum and PBS control. After the addition of avidin, three separate samples of 100 Tl of one of three concentrations of B-BSA, 1.900, 0.500 and 0.125 mg/ml was added giving a final B-BSA concentration of 90, 24, or 6 Tg/ml. Solutions were allowed to incubate at room temperature for 15 minutes, then 100 Tl volumes were transferred in triplicates to a 96-well microplate (Nunc, Immulon 4 microplate). Following a 45 min room temperature incubation, wells were washed 5 times with distilled water. Casein blocker (Pierce, Rockford, IL) was then added to wells (280 Tl/well) and blocking allowed for 40 min. The rinse procedure followed, then the addition of horseradish peroxidase conjugated biotin. A 20 min incubation followed, then a rinse, and the addition of 3, 3', 5, 5' tetramethyl benidine (TMB substrate, Pierce, Rockford, IL). Absorbances were read with a Dynatech MR580 (Chantilly, VA) microplate reader every 5 min for 40 min. A second assay was conducted using the same procedure except for an extended incubation time to 90 min after the addition of blood or serum containing avidin and B-BSA.

Serial dilution of avidin to determine lowest levels detectable by ELISA

To detect the lowest levels of avidin in serially diluted samples of PBS beginning with a stock solution which had an avidin concentration of 7.5 Tl/ml. Twelve dilutions were made from the stock solution, each cutting the concentration of avidin in the previous solution by half. The assay was run in duplicate sets of avidin dilutions in PBS to test for differences between the use, or lack of 50 Tl/ml Tween 20 (Sigma Chemical Company, St. Louis, MO) in the wash buffer (PBS). In addition, a comparison between avidin and no avidin, with or without tween in the wash buffer was conducted. Each dilution was run in duplicate wells.

Serial dilution of avidin in serum to determine nonspecific binding

Two serial dilution procedures were completed using ELISA. The first, was a serial dilution of normal Atlantic salmon serum with 7.5 Tl/ml of avidin added in PBS. Normal serum was spiked with avidin and diluted by a factor of ten from 1:0 to 1:10,000. Each dilution was added to microplate wells in duplicate. After pipetting 100 Tl of solution into microplate wells, the plates were incubated for 45 min at room temperature. Plates were then washed five times with distilled water and blocked with casein for 40 min. After incubation, 100 Tl biotin conjugated with horseradish peroxidase (HRP, Pierce, Rockford, IL) as the second antibody, was added to each well either with or without Tween 20 (50 Tl Tween/1.5 ml biotin-HRP) and the plate incubated for 20 min. The plate was then washed as before, and TMB substrate added. Absorbance readings were taken immediately, and at 5 min intervals for 40 min. In the second assay, serum dilutions to 1:10,000 were tested in paired groups. These included normal serum with and without Tween, serum with avidin (7.5 Tl/ml), with and without Tween 20; and serum with avidin, with and without Tween with the addition of the avidin-biotinylated HRP enzyme complex (ABC, Pierce, Rockford, IL). The ABC reacts with substrate to form a chromogenic product detectable with the Dynatech ELISA reader.

Molecular marking of Atlantic salmon fry

Atlantic salmon fry were immunized in a 3.1 Tg/ml B-BSA bath just prior to swim-up in May of 1996. The fish were immersed in the bath for 5 minutes at 8.3 ^{0}C. After the bath treatment with B-BSA, the fish were reared in 8-10 ^{0}C

water in 40 (two half tanks) to 80 liter glass tanks with flow through water supplied at 4 L/minute. Fish were anesthetized in methane tricaine sulfonate and blood was drawn from the caudal peduncle from 10 fish per treatment group. Blood was refrigerated overnight, centrifuged at 13,000 rpm for 8 minutes the following day, and serum drawn off and stored frozen for ELISA. For ELISA the serum was diluted 1:1 in PBS and pipetted 100 Tl per well into duplicate microplate wells and incubated overnight at 4 ^{0}C. The plate was washed, blocked, incubated with ABC, washed, substrate added and read as above.

Statistical analysis

Statistical analysis was calculated from single mean values derived from all duplicate pairs. We used one way analysis of variance to show significant differences between biotin-avidin formation in blood and in serum. Serial dilutions were plotted to show differences among absorbances resulting from dilutions. The decision rule for all statistical tests was set at p=0.05.

Results

\The first experiments showed that B-BSA would bind to avidin in both blood and serum forming an avidin-B-BSA complex (AV-B-BSA) in both medias. AV-B-BSA bound more readily in serum than in blood as shown (Table 1) by ELISA absorbance following addition of the same concentrations of avidin (7.5 Tg/ml) and B-BSA (6, 24, and 90 Tg/ml). Absorbance values in blood were significantly lower than those found in serum. Absorbance values for the AV-B-BSA complex in serum were higher than those in blood and significantly different among B-BSA levels with highest absorbances in the 6 Tg/ml B-BSA concentration after 20 minutes incubation time (assay #1), and higher for 90 Tg/ml B-BSA concentration in the second assay (90 minute incubation, Table 1). Additional incubation time allowed for the AV-B-BSA complex to form in a dose dependent relationship in serum (Table 1, assay #2) and was detectable as low as 6 Tg/ml B-BSA and 7.5 Tg/ml avidin.

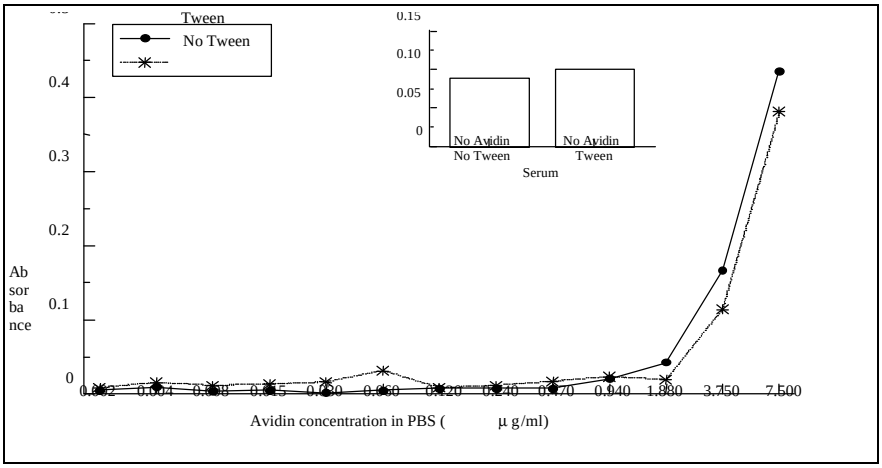
A standard curve for avidin was completed showing that for the AV-B-BSA-enzyme complex, the lowest detectable level of avidin was between 3.75 and 1.88 Tg/ml when using serial dilution of avidin in PBS (Fig. 1). Negative controls using serum are also shown on Fig. 1 (inset) indicating negative control values for serum are similar to those produced by B-BSA-avidin in PBS (No

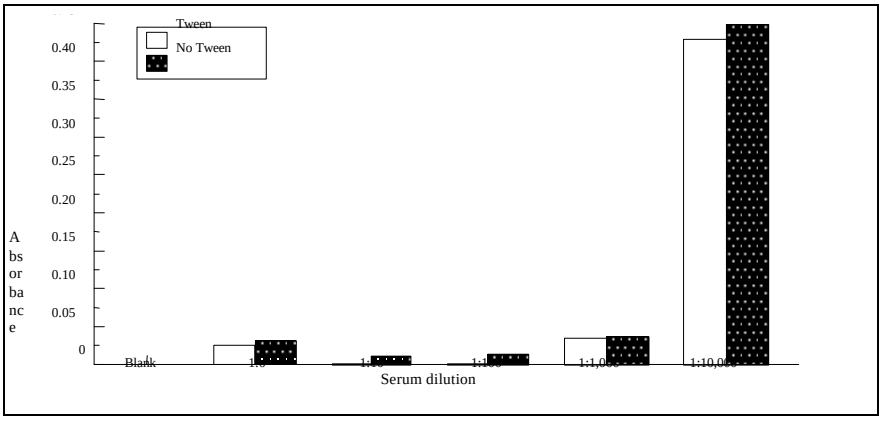
avidin-no Tween and No avidin-Tween groups) and that absorbance values (Inset, Fig. 1) were shown for avidin concentrations in PBS of 1.88 Tg/ml avidin or lower.

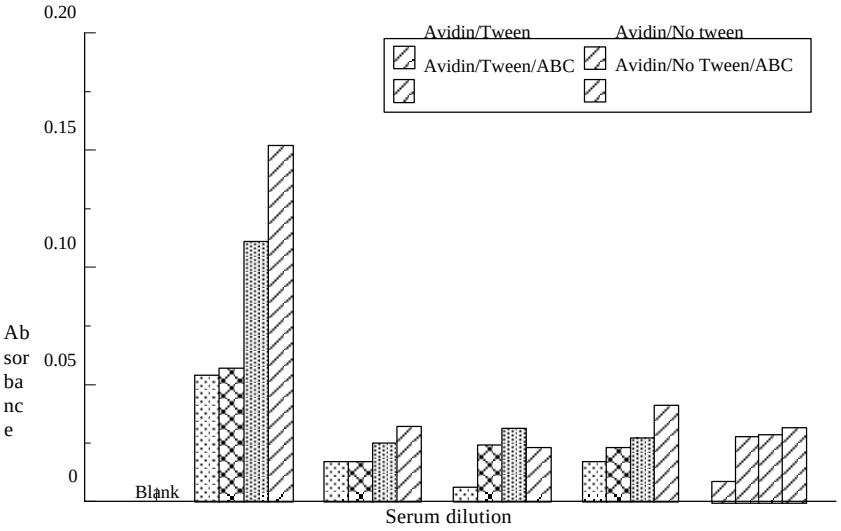
Serial dilution of control serum was conducted to determine the dilution rate where nonspecific protein binding interfered with the assay. The extent of nonspecific protein binding is contrasted with normal assay serum dilutions of 1:0 or 1:1, serum:PBS. We repeated an initial assay which showed that nonspecific protein binding did not occur until serum was diluted by 1:10,000. A second assay gave the same result as the first showing that nonspecific protein binding occurs only when serum is diluted at 1:10,000 in PBS (Fig. 2).

Table 1. Mean (n = 6, with standard error of the mean, SEM) ELISA assay absorbances of 6, 24, or 90 Tg/ml biotinylated bovine serum albumin (B-BSA) added to Atlantic salmon blood and serum containing avidin. Values with different superscripts are significantly different ($p < 0.05$).

		Blood			Serum					
Assay	Incubation (minutes)	B-BSA (Tg/ml)	Absorbance	SEM	B-BSA (Tg/ml)	Absorbance	SEM			
1	20	90	0.030^{c}		90	0.008				
		90	0.239^{ab}		90	0.027				
		24	0.034^{c}		24	0.006		24	0.186^{b}	0.017
		6	0.049^{c}		6	0.012		6	0.275^{a}	0.035
2	90	90	0.076^{cd}	0.004	90	0.646^{a}	0.008			
		24	0.125^{c}	0.019	24	0.609^{ab}	0.020			
		6	0.065^{d}		6	0.005		6	0.576^{b}	0.016

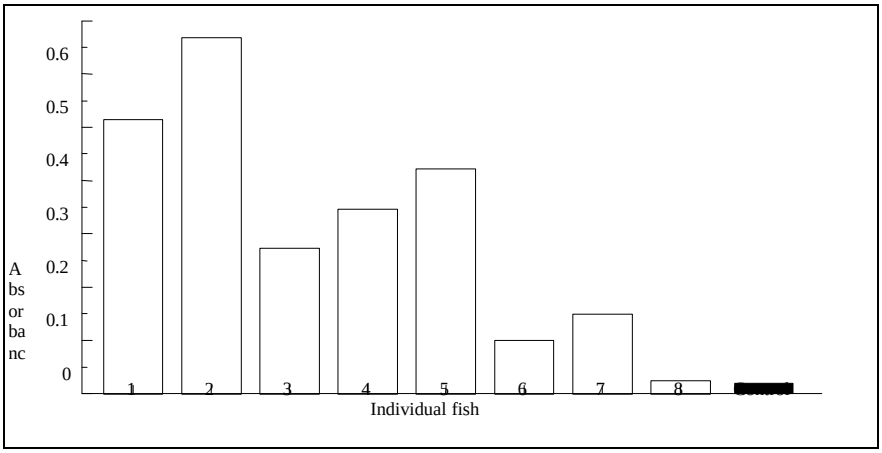

Tween
No Tween
0.4
0.3
0.2
0.1
0
Absorbance
0.15
0.10
0.05
0
No Avidin
No Tween
No Avidin
Tween
Serum
0.002
0.004
0.940
1.880
3.750
7.500
Avidin concentration in PBS (µ g/ml)


Tween
No Tween
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
Absorbance
Blank
1:1,000
1:10,000
Serum dilution



To compare the effects of Tween 20 in wash buffer, and the use of the ABC in assay development, we conducted serial dilutions of Atlantic salmon serum with the assay reagents (Fig. 3). The first pair of data on Fig. 3 show absorbance of constant concentrations of avidin without the use of ABC showing low values. The second paring of AV-B-BSA-enzyme complex (Fig. 3) comparing Tween and no Tween in the wash buffer gave best results at 1:0 dilution. These absorbances were similar to those of serum from marked fish. These final two sets of bars on Fig. 3 also show that a specific assay for AV-B-BSA provides positive results over the dilution rate of our normal assay of 1:1 serum dilution in PBS over nonspecific protein binding in the ELISA plate.

Of the eight salmon checked for B-BSA 14 months after immunization, we found 7 of 8 with an acceptable titer of more than a 2:1 absorbance ratio above the absorbance of the negative control serum (Fig. 4). One fish had a titer less than twice the absorbance of negative control serum. Negative control serum absorbance was 0.025 and the negative sample 0.037. All positive absorbances of individual fish 14 months after immunization had titers of 0.100 to 0.688.



Discussion

Results of this study show that the B-BSA-avidin-enzyme complex forms a readable mark in Atlantic salmon blood or serum. By using this method we can then produce a retrievable biochemical mark, and when many marks are applied we can identify different groups of fish. Lowest detectable levels of *AV-B-BSA* complex *in vitro* in serum provides a basis for determining dose to bath treatments for fish, resulting in a more sensitive *in vivo* recognition of the stock identification marker. This method is intended for use with larval fish, or those smaller than fish which cannot accept juvenile fish tagging methods such as fin clips, hot or cold brands, coded wire tags, elastomer tags, or fluorescent sprays, etc. (Bandow 1987, Negus et al. 1990, Younk and Cook 1991, Thedinga and Johnson 1995, Moffett et al. 1997, Dussault and Rodriguez 1997). Retrieval of the mark is also intended to be simple, and without necessity for lethal sampling methods as is the case with otolith marks or rare earth element markers (Younk and Cook 1991, Nielsen 1992, Schroder et al. 1996). Application procedures for this present method are also simpler than those for calcein fluorescent markers, which may require up to 48 hours exposure to provide a successful mark (Gelsleichter et al. 1997, Mohler 1997). The biochemical marking method presented here is useful as a batch marking method with enough different tags could be used to mark small groups such as families, but may not be as useful as current genetic methods which can be used for genetic analysis of families.

However, with genetics markers, a search for suitable alleles must first take place, and both parents and progeny sampled. In addition, genetics procedures require the use of sophisticated equipment, the use of genetics expertise to interpret results (Nielsen 1992), and there is a reasonably high cost for the evaluation of genetic makers.

It has also occurred to us that the ability of pre-feeding larval salmonid fish to take up and process an antigen tagged as a stock identification marker appears probable since the humoral immune system is functioning by the time the fish begin feeding. The Atlantic salmon has membrane IgM at first feeding, indicating a functioning humoral immune system (Ellis 1977) and the chum salmon (*Oncorhynchus keta*) has rapidly increasing IgM concentrations at first feeding (Nagae et al. 1993). The rainbow trout (*Oncorhynchus mykiss*) is shown to have peak IgM at hatching (Castillo et al. 1993) and able to produce a titer at one month post-hatch (Tatner 1986). Because the immune system is functional, the use of immunization in an antigen then produces a possible means of marking larval fish.

Results of this present study show that Atlantic salmon fry immunized prior to first feeding can retain recognition of their history of exposure to an antigen immunization for over one year. These results coupled with the *in vitro* assays performed in this study show the possibilities of marking salmonid fish with tagged antigens.

Literature Cited

Bandow, F. 1987. Fluorescent pigment marking of seven Minnesota fish species. Minnesota Department of Natural Resources, Division of Fish and Wildlife, Investigational Report #393.

Castillo, A., C. Sanchez, J. Dominguez, S. L. Kaattari, and A. J. Villena. 1993. Ontogeny of IgM and IgM-bearing cells in rainbow trout. Developmental and Comparative Immunology 17:419-424.

Dussault C. and M. A. Rodriguez. 1997. Field trials of marking stream salmonids by dye injection and coded-wire tagging. North American Journal of Fisheries Management 17:451-456.

Ellis, A. E. 1977. Ontogeny of the immune response in *Salmo salar*. Histogenesis of the lymphoid organs and appearance of membrane immunoglobulin and mixed leucocyte reactivity. pages 225-231 *in* J. B. Solomon and J. D. Horton, editors, Developmental Immunobiology. Elsevier/North Holland Biomedical Press, Amsterdam, The Netherlands.

Moffett, I. J. J. , W. W. Crozier, and G. J. A. Kennedy. 1997. A comparison of five external marks for Atlantic salmon, *Salmo salar* L. Fisheries Management and Ecology 4:49-53.

Mohler, J W. 1997. Immersion of larval Atlantic salmon in calcein solutions to induce a non-lethally detectable mark. North American Journal of Fisheries Management 17:751-756.

Nagae, M., H. Fuda, A. Hara, H. Kawamura, and K. Yamauchi. 1993. Changes in serum immunoglobulin M (IgM) concentrations during early development of chum salmon (*Oncorhynchus keta*) as determined by sensitive ELISA technique. Comparative Biochemistry and Physiology 106A:69-74.

Negus, M. T., D. A. Belford, and S. E. Colvin. 1990. Long-term retention of fluorescent pigment marking of chinook salmon. Minnesota Department of Natural Resources, Section of Fisheries, Investigational Report # 402.

Nielsen, L. A. 1992. Methods of Marking Fish and Shellfish. American Fisheries Society, Special Publication 23, Bethesda, MD.

Schroder, S. L., E. C. Volk, C. M. Knudsen, and J. J. Grimm. 1996. Marking embryonic and newly emerged salmonids by thermal events and rapid immersion in alkaline-earth salts. Bulletin of the National Research Institute for Aquaculture, Supplement 2:79-83.

Tatner, M. F. 1986. The ontogeny of humoral immunity in rainbow trout, *Salmo gairdneri*. Veterinary Immunology and Immunopathology 12:93-105.

Thedinga, J. F. and S. W. Johnson. 1995. Retention of jet-injected marks on juvenile coho and sockeye salmon. Transactions of the American Fisheries Society 124:782-785.

Younk, J. A. and M. F. Cook. 1991. Fluorescent chemical marking of walleye larvae with a selected literature review of similar investigations. Minnesota Department of Natural Resources, Section of Fisheries, Investigational Report, # 408.

THE EFFICACY OF BATH TREATMENTS AND USE OF DIMETHYL SULFOXIDE IN APPLYING BIOCHEMICAL MARKERS FOR STOCK IDENTIFICATION

William F. Krise
United States Geological Survey
Research and Development Laboratory
Rural Route #4, Box 63
Wellsboro, PA 16901

and John L. Sternick
Biology Department, Grant Science Center
Mansfield University
Mansfield, PA 16933

Abstract

This study was one of a series to determine if exposure of salmonids with a protein mark provides a suitable method for marking small fish for stock identification. Atlantic salmon (*Salmo salar*), mean length 104 mm, were exposed to 10-minute baths of 5, 10, and 20 µg/ml biotinylated bovine serum albumin (B-BSA) and a second group was injected with 1.0, 2.5, 5.0, and 10.0 µg B-BSA. The purpose of this study was to determine if mark retention were similar between the bath and injection applications and which bath concentrations would be most effective in producing a high mark concentration in fish. In a second experiment, salmon were given baths of 10 µg/ml B-BSA with one of four dimethyl sulfoxide (DMSO) concentrations, 0, 1, 2, or 4% DMSO to determine if antigen uptake was increased by DMSO in the bath. Blood samples were collected from exposed fish 10, 30, 40, and 90 days after exposure. Serum was used in ELISA to determine concentration levels. There were no significant differences in B-BSA absorbance between salmon bath or injection rates, or among any of the bath or injection treatments on any sample date. There was no advantage in using DMSO at concentrations indicated

above. We recommend further study on the longevity of antibody detection in salmonids immunized as fry to develop a fry marking method.

Introduction

Fish traditionally have been exposed to protein either via an injection, or by immersion in a bath treatment. Comparison of titer from both methods gives an indication of the efficiency of protein delivery to the fish. For delivery of protein mark to fish too small for injections, the use of a bath treatment is needed. Successful bath exposure result in high protein mark concentration about 30 days post immunization, with high immune titers lasting about 75 days (Anderson et al. 1979). Comparison of the two exposure methods, intraperitoneal (IP) injection and bath immersion, during 90 days post-treatment, gives an indication of effectiveness of exposure treatments.

In this study, we observed mark retention in Atlantic salmon (*Salmo salar*) juveniles through 90 days post exposure. The purpose of our study was to determine effectiveness of protein bath and injection treatments in juvenile Atlantic salmon for application in marking fry with tagged proteins. Marking of fry less than 25 mm long requires the use of bath exposure, rather than injection, primarily due to fish size.

Materials and Methods

Atlantic salmon parr, with a mean length of 104 mm and a mean weight of 13.0 g, were randomly separated into seven groups of 42 fish each, and each group was given a different exposure either by injection or by bath immersion. Four IP treatments were given at 10, 25, 50, and 100 µg biotinylated bovine serum albumin (B-BSA) in 0.1 ml sterile distilled water. Thus, protein mark was administered at rates of 1.0, 2.5, 5.0 and 10.0 µg per fish. Fish were anesthetized in methane tricaine sulfonate, given the IP injection with a 26 gauge needle, and placed into separate rearing units for each treatment group. Three groups of fish were immersed in bath treatments at one of three B-BSA protein concentrations: 5, 10, and 20 µg/l. Bath treatments were given in polypropylene tubs with the fish held in nets within the tubs. Fish were lightly anesthetized prior to immersion to reduce stress. Immersion lasted 7 minutes, then the fish were placed into separate rearing units.

Fish given DMSO baths (0, 1, 2, and 4% DMSO) were lightly anesthetized, and placed into a polypropylene tub, held in a net and immersed in the antigen bath for 8 minutes. Once the bath was complete, the fish were placed into the same rearing tanks as the fish in the above study and held for 90 days, with blood sampled from salmon on days 10, 30, 40, and 90. Samples from all tests were collected in the same manner.

Blood samples from fish in both tests were collected from anesthetized fish (n=10 per treatment) 10, 30, 40, and 90 days after administration of treatments. Blood was refrigerated 4-6 hours, centrifuged for 8 minutes at 13,000 rpm, and serum removed and stored frozen until assayed for B-BSA. Serum mark was measured by ELISA.

The ELISA procedure for all tests included the use of positive control groups of phosphate buffered saline (PBS) with B-BSA added at one of five concentrations: 1.90, 1.00, 0.50, 0.25, and 0.10 mg/ml. Serum or controls were diluted in PBS to 100 µl, then diluted 1:1 in PBS before addition to the wells of a 96-well microplate (100 µl/well) and incubated in a refrigerator overnight. Absorbances were adjusted to the ratio of PBS added to serum before the 1:1 dilution in PBS. After warming to room temperature the next day, serum and controls were removed and plates washed with PBS five times. Plates were then blocked with 280 µl per well of casein blocking buffer (Pierce, Rockford, IL) and incubated for 45 min. The previous wash procedure was repeated with 50 µl Tween 20 added to the PBS. After washing, 2 drops of ABC complex (Pierce, Rockford, IL) were added to each well (80 µl) and plates were incubated for 20 min. Plates were washed again five times with PBS-Tween, substrate (TMB substrate, Pierce, Rockford, IL) added, and plates read at 5 minute intervals. The twenty-minute reading was selected for providing peak readings. Absorbance was read on a Dynatech MR 580 (Chantilly, VA) microplate reader.

We used one-way analysis of variance to determine differences among means of each treatment and the Tukey's multiple comparison of means to determine which treatment means were significantly different at the $p=0.05$ level of significance. Due to differences expected among ELISA plates at each assay (conducted on different days), we did not attempt to compare statistical differences using two-way analysis of variance. All sample sizes were 8-10 fish per treatment.

Results

Absorbance from serum samples collected 10-days post exposure showed high variability within treatment groups and mean absorbances were statistically the same for fish given either antigen injections or bath treatments (Table 1). At this stage, several of the fish tested had low response to the antigen regardless of its presentation or concentration.

Table 1. Mean Atlantic salmon serum titers (SEM) using ELISA absorbance to biotinylated bovine serum albumin (B-BSA) following bath exposure or intraperitoneal injection.

	Sample date (days post treatment)			
Bath/concentration	10	30	40	90
5 µg/ml	0.124(0.078)	0.165(0.038)	0.124(0.082)	0.212(0.039)
10 µg/ml	0.209(0.085)	0.178(0.060)	0.337(0.098)	0.300(0.138)
20 µg/ml	0.106(0.049)	0.154(0.067)	0.159(0.044)	0.276(0.053)
Injection (per fish)				
1.0 µg B-BSA	0.240(0.070)	0.170(0.021)	0.237(0.097)	0.248(0.091)
2.5 µg B-BSA	0.203(0.062)	0.304(0.063)	0.186(0.058)	0.220(0.103)
5.0 µg B-BSA	0.093(0.015)	0.272(0.073)	0.179(0.057)	0.314(0.148)
10.0 µg B-BSA	0.114(0.020)	0.239(0.067)	0.165(0.081)	0.201(0.053)
Negative control	0.015(0.003)			
0.015(0.005)				
Positive control	0.770(0.001)	0.641(0.002)	0.636(0.001)	0.387(0.002)
		0.584(0.010)	0.610(0.002)	0.396(0.001)
		0.647(0.002)	0.651(0.014)	

Absorbance values 30 days post-exposure represented the probable peak of mark detection but these absorbance values do not appear significantly different among any of the treatments (Table 1). Variability among individual fish was lower in the 10 to 40-day samples than in the 90-day post-exposure sample.

The 40-day sample showed that the mean absorbance levels were either similar or slightly lower than those from fish 30-days post-exposure. Relationships with treatments remained the same as in previous treatments (Table 1).

The 90-day sample yielded similar results to those of the previous sample dates. There were no significant differences among treatments for either serum concentrations of B-BSA to bath or injection treatments (Table 1). Variability among marked individuals was highest in this sample set. Concentrations from all levels of mark dosage yielded similar mean serum concentrations between 10 and 20 times those of negative control serum.

We found no significant differences among Atlantic salmon blood concentrations of B-BSA or BSA/avidin complex due to the use of DMSO in the exposure bath on any of the sampling dates tested (Table 2). Reasonable test results were obtained through 90 days after exposure, but that variability in positive responses of individuals resulted in non-significant differences among these treatments and times.

Table 2. Mean Atlantic salmon serum titers (SEM) to 10 mg/L baths of biotinylated bovine serum albumin with 0, 1, 2, or 4% dimethyl sulfoxide (DMSO) in the exposure bath.

	Sample day (post exposure)			
DMSO (%)	10	30	40	90
0	0.241(0.114)	0.987(0.240)	0.327(0.183)	0.666(0.142)
1	0.703(0.239)	0.587(0.159)	0.585(0.271)	0.769(0.224)
2	0.762(0.424)	0.786(0.208)	0.739(0.170)	0.680(0.290)
4	0.604(0.138)	0.777(0.207)	0.915(0.175)	0.581(0.170)
Negative control	0.021	0.028	0.027	0.142(0.092)
Positive control	0.536	0.462	0.512	0.358(0.001)
	0.552	0.541		0.370(0.010)
	0.574			
	0.549			

Discussion

Results of this study indicate that juvenile Atlantic salmon were able to achieve similar concentrations of B-BSA over a range of injection rates and bath treatment concentrations. Effective bath treatments have been given with as little as 2 minute exposure time (Anderson et al. 1979, Zapata et al. 1987) with varied dosages of antigen from 1 to 1,000 µg/ ml. Anderson et al. (1979) found that a 10 µg/ml concentration of *Yersinia ruckeri* 0-antigen was sufficient to produce a response. The 7-8 minute exposure time to BSA used in the present study was long enough to produce a high mark concentration of B-BSA in Atlantic salmon.

Atlantic salmon are immunocompetent at the end of the yolk-sac absorption period with membrane IgM present on lymphocytes (Ellis 1977). Similarly, other salmonid species are immunocompetent at the end of the yolk absorption period, such as the chum salmon (*Oncorhynchus keta*, Nagae et al. 1993), or the rainbow trout (*Oncorhynchus mykiss*, Tatner 1986). Immunocompetence at the pre-feeding stage in salmonids allows the use of protein bath treatments for marking salmonid fry and allows the marked fish group to be identified later. The information from this study should be applicable for establishing exposure concentrations for marking of fry.

Since there are several routes of mark entry into the fish with bath treatments, we feel that fry or alevins would be able to produce an appropriate response to the challenge in a bath. Assuming that the humoral immune response is functional in salmonid fry by the time fish begin exogenous feeding (Ellis 1977, Atlantic salmon; Nagae et al. 1993, chum salmon; Fuda et al. 1991, masu salmon (*Oncorhynchus masou*); Tatner 1986, and Castillo et al. 1993, rainbow trout), then the routes of antigen entry into a fry are important in the fish's uptake and processing of antigen. Antigen routes of entry could include the gills, skin and the gastrointestinal tract. Antigen entry through the gills was shown for juvenile Atlantic salmon in a 2 minute bath of *Yersinia ruckeri* 0-antigen (Zapata et al. 1987) and for rainbow trout in a 1 hour bath of *Flavobacterium branchiophilium*). Ototake et al. (1996) found that rainbow trout take up antigen through the skin. They used BSA and exposed 15 g juvenile trout to 2% concentrations of BSA in 3 minute baths. Lastly, Robohm and Koch (1995) showed that a major route of uptake of antigen occurs through the gastrointestinal tract. The authors plugged the esophagus of fish and showed

greatly reduced uptake of *Clostridium botulinum* toxin. In our bath exposures, Atlantic salmon could take up and process BSA in any or all of these routes.

Some variation in mark uptake and processing experienced in the fish in our study could be explained by the fact that the fish were anesthetized to administer the mark, or that genetic differences among fish could result in different mark retention levels. Lobb (1987) found that channel catfish (*Ictalurus punctatus*) had depressed antigen uptake due to anesthesia. Genetic differences in hemolytic or lysozyme activity have been shown for rainbow trout and Atlantic salmon (Roed et al. 1990, Roed et al. 1992, Roed et al. 1993, and Lund et al. 1995). Differences among genetics of individual fish in our study could have led to their high variation in response. However, we feel that different individual fish responses to anesthesia were probably the largest cause of variability among treatment groups.

We found that juvenile Atlantic salmon could take up B-BSA as a mark effectively through bath treatments ranging from 5 to 20 µg/ml. Their mark concentrations were statistically the same as those from fish given IP injections of B-BSA. The use of DMSO in bath treatments did not significantly improve the uptake of the mark. Immunization of Atlantic salmon with an antigen bath may provide an effective method of marking small fish for stock identification.

References

Anderson, D. P., O. W. Dixon, and B. S. Roberson. 1979. Kinetics of the primary immune response in rainbow trout after flush exposure to *Yersinia ruckeri* O-antigen. Devel. and Comp. Immunol. 3:739-744.

Castillo, A., C. Sanchez, J. Dominguez, S. L. Kaattari, and A. J. Villena. 1993. Ontogeny of IgM and IgM-bearing cells in rainbow trout. Devel. and Comp. Immunol. 17:419-424.

Ellis, A. E. 1977. Ontogeny of the immune response in *Salmo salar* histogenesis of the lymphoid organs and appearance of membrane immunoglobulin and mixed leucocyte reactivity. Pages 225-231 *In* J. B. Solomon and J. D. Morton, editors. Developmental Immunobiology. Elsevier/North-Holland Medical Press, Amsterdam.

Fuda, H., K. Soyano, F. Yamazaki, and A. Hara. 1991. Serum immunoglobulin M (IgM) during early development of masu salmon (*Oncorhynchus masou*). Comp. Bioch. Physiol. 99A:637-643.

Lobb, C. J. 1987. Secretory immunity induced in catfish, *Ictalurus punctatus*, following bath immunization. Devel. and Comp. Immunol. 11:727-738.

Lund, T., T. Gjerdrem, D. M. Eide, H. J. S. Larsen, and K. H. Roed. 1995. Genetic variation in immune parameters and associations to survival in Atlantic salmon. J. Fish Biol. 46:748-758.

Nagae, M., H. Fuda, A. Hara, H. Kawamura, and K. Yamauchi. 1993. Changes in serum immunoglobulin (IgM) concentrations during early development of chum salmon (*Oncorhynchus keta*) as determined by sensitive ELISA technique. Comp. Bioch. Physiol. 106A:69-74.

Ototake, M., G. K. Iwama, and T. Nakanishi. 1996. The uptake of bovine serum albumin by the skin of bath-immunized rainbow trout *Oncorhynchus mykiss*. Fish and Shellfish Immunol. 6:321-333.

Robohm, R. A. and R. A. Koch. 1995. Evidence for oral ingestion as the principal route of antigen entry in bath-immunized fish. Fish and Shellfish Immunol. 5:137-150.

Roed, K. H. 1990. The genetic influence on serum haemolytic activity in rainbow trout. Aquaculture 85:109-117.

Roed, K. H. 1992. Genetic variation in haemolytic activity in Atlantic salmon (*Salmo salar* L.). J. Fish Biol. 40:739-750.

Roed, K. H., K. T. Fjalestad, and A. Stromsheim. 1993. Genetic variation in lysozyme activity and spontaneous haemolytic activity in Atlantic salmon (*Salmo salar*). Aquaculture 114:19-31.

Tatner, M. F. 1986. The ontogeny of humoral immunity in rainbow trout, *Salmo gairdneri*. Vet. Immunol. Immunopath. 12:93-105.

Zapata, A. G., M. Torroba, F. Alvarez, D. P. Anderson, O. W. Dixon, and M. Wisniewski. 1987. Electron microscopic examination of antigen uptake by salmonid gill cells after bath immunization with a bacterium. J. Fish Biol. 31 (Supplement A):209-217.

TRANSPORT OF LACTATE STUDIED IN RAINBOW TROUT WHITE MUSCLE USING GIANT SARCOLEMMAL VESICLES

Karen Laberee
Department of Zoology
University of Western Ontario
London, ON Canada N6A 5B7
klaberee@julian.uwo.ca

C. Louise Milligan
Department of Zoology
University of Western Ontario
London, ON Canada N6A 5B7
519-661-3869/519-661-2104/milligan@julian.uwo.ca

Introduction

Exhaustive exercise in salmonids such as the rainbow trout, *Onchorynchus mykiss*, has been the focus of several studies over the last decade. The fate of lactic acid, which is produced during glycolysis, has been of particular interest since it is not simply discarded by the white muscle for conversion elsewhere in the body. It has been shown that the majority of lactic acid, or lactate and protons as it is found at physiological pH, is retained within the white muscle for upwards of 6 to 8 hours following production (Milligan and Wood, 1986). This is in sharp contrast to the situation in mammals where pre-exercise levels of both lactate and glycogen are reached within an hour. It is thought that lactate in fish white muscle is converted to glycogen via *in situ* glyconeogenesis, possibly by the reversal of pyruvate kinase (Milligan, 1996; Moyes et al., 1992). Furthermore the results from studies done by Turner and Wood (1983) and Milligan and Girard (1993) indicate that some of the lactate that leaves the muscle is taken back up by the white muscle. As well it has been demonstrated that blood-borne glucose does not contribute significantly to the glycogen restoration in the white muscle (Pagnotta and Milligan, 1991). Thus it would appear that lactate is the key substrate for glycogen resynthesis and as such its passage across the sarcolemma should be regulated. It was our objective to study

this transport of lactate across the sarcolemma using sarcolemmal vesicles isolated from trout white muscle.

Sarcolemmal vesicles have been used by mammalian physiologists to study the transport of lactate, glucose and other metabolites across the sarcolemmal membrane. This preparation is advantageous as the experimental conditions on both sides of the membrane can be controlled. Metabolism of the substrate under study is also not of concern when using sarcolemmal vesicles as the cell's organelles are absent. As well this technique focuses the transport to one barrier, the sarcolemma. Juel (1991) developed a technique using collagenase to create giant (1-40μm) vesicles that are almost exclusively right-side out. This giant vesicle technique has been used by Bonen and colleagues to study the transport of lactate, glucose and fatty acids in mammalian muscle tissue. We have adapted the technique of Juel (1991) to the white muscle of rainbow trout and have produced viable and harvestable giant sarcolemmal vesicles, although in much smaller yields than with mammalian muscle.

With these giant trout sarcolemmal vesicles we have begun to characterize the nature of the transport of lactate into the white muscle. The uptake of lactate by the sarcolemmal vesicles was shown to be linear within he 20 to 30 second range (Figure 1). A transport period of 30 seconds was chosen for subsequent transport studies in order to optimize both rate and yield of uptake. The transport of lactate into the vesicles showed saturation kinetics when the external concentration of lactate was varied (Figure 2a.). This data has been replotted in a Lineweaver-Burke plot, and a K_m of 28.5 mM was obtained (Figure 2b). This is not far from the K_m of 20.9 mM for lactate obtained by Juel (1991) in mammalian zero-trans sarcolemmal vesicle studies. The upper limit of blood lactate following exhaustive exercise is approximately 20 mM (Milligan, 1996). As well we obtained a V_{max} of 35.71 nmol/mg·min which is also in the same range as the V_{max} of 28.8 nmol/mg·min determined by Juel (1991) in his zero-trans study. At present we are attempting to obtain a clearer picture of these transport phenomena using the known lactate inhibitors α-cyano-4-hydoxycinnamic acid (CIN) and p-chloromercuriphenylsulfonic acid (PCMBS), as well as likely competitors for a transporter, pyruvate and D- lactate.

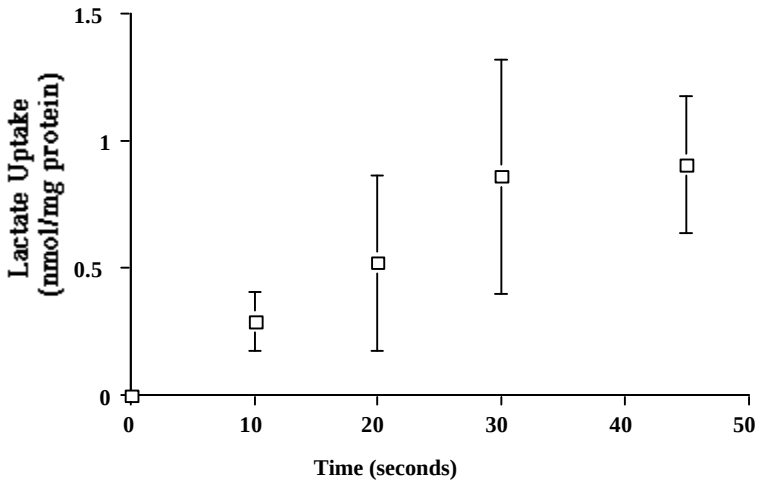


Figure 1. The uptake of lactate by trout white muscle sarcolemmal vesicles over the period of 45 seconds. The extracellular cold lactate concentration was 1 mM and 0.5 μCi of universally labelled ^{14}C lactate was added per reaction tube. Means ± 1 SEM, N=4 for each time.

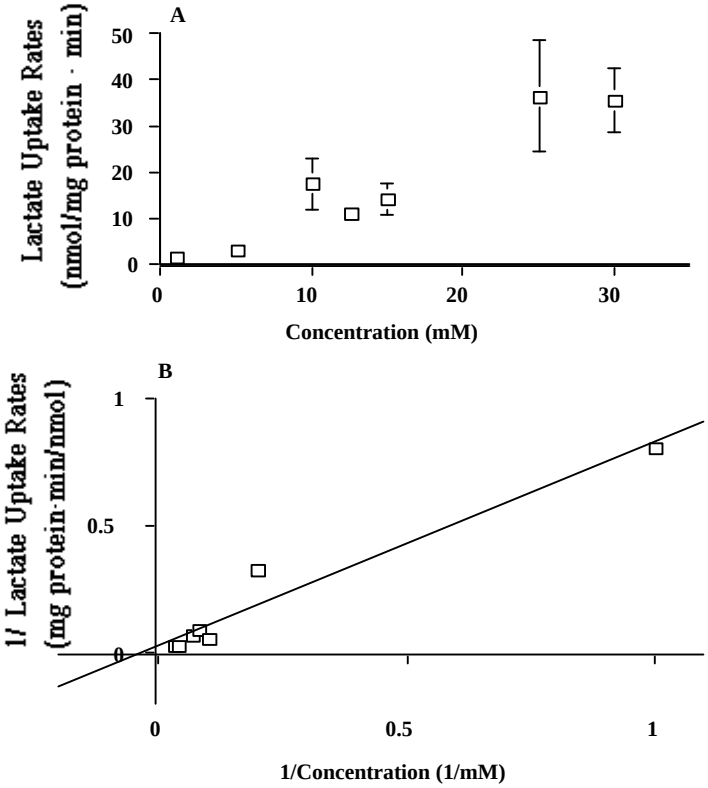


Figure 2A. The rates of lactate uptake over 30 seconds at different concentrations of external lactate by trout white muscle sarcolemmal vesicles. **B.** These rates expressed as a Lineweaver-Burke plot. The equation of the line was found to be $y=0.798x +0.028$, $r^2=0.952$. The K_m was calculated to be 28.5 mM and the V_{max} to be 35.71 nmol/mg protein·min. 0.5 μCi of universally labelled ^{14}C lactate was added per reaction tube. Means±1 SEM. N=6 for the 1, 10 and 25 mm concentrations; N=4 for the 5 mM concentration, and N=3 for the 15 and 30 mM concentrations.

Acknowledgements

The authors wish to thank Dr. Arend Bonen, University of Waterloo, for his time and generosity in teaching the mammalian giant sarcolemmal vesicle technique. This work was supported by an NSERC grant to CLM.

References

Juel, C. 1991. Muscle lactate transport studied in sarcolemmal giant vesicles. Biochim. Biophys. Acta 1065: 15-20.

Milligan, C. L. 1996. Metabolic recovery from exhaustive exercise in rainbow trout.Comp. Biochem. Physiol. 113A(1): 51-60.

Milligan, C. L. and S. Girard 1993. Lactate metabolism in rainbow trout. J. Exp. Biol.180: 175-193.

Moyes, C. D., P. M. Schulte and P.W. Hochachka. 1992. Recovery metabolism of trout white muscle: role of mitochondria. Am. J. Physiol. 262: R295-R304.

Pagnotta, A. and C. L. Milligan 1991. The role of blood glucose in the restoration of muscle glycogen during recovery from exhaustive exercise in rainbow trout (*Oncorhynchus mykiss*) and winter flounder (*Pseudopleuronectes americanus*). J. Exp. Biol. 161: 489-508.

Turner, J. D. and C. M. Wood 1983. Factors affecting lactate and proton efflux from pre-exercised, isolated-perfused rainbow trout trunks. J. Exp. Biol. 105: 395-401.

TEMPERATURE AND FLOW EFFECTS

ON JUVENILE SALMON

SWIMMING AND MUSCLE DYNAMICS:

RESEARCH IN PROGRESS

Shana M. Katzman
Department of Wildlife, Fish, and Conservation Biology
University of California, Davis, CA 95616
(530) 752-8659, FAX (530) 752-4154,
email: smkatzman@ucdavis.edu

Joseph J. Cech, Jr.
Department of Wildlife, Fish, and Conservation Biology
University of California, Davis, CA 95616
(530) 752-3103, FAX (530) 752-4154,
email: jjcech@ucdavis.edu

Introduction

Dams and reservoirs have interrupted historical paths for migrating salmon in many California rivers. Associated water quality problems (including decreased flows and increased temperatures) and predation problems on young migrating salmon have exacerbated their populational declines (NRC 1996). Salmon hatcheries will probably constitute one tool in the rehabilitation of depleted salmon stocks, but questions remain concerning optimal release times of smolting salmon for their successful emigration to the ocean. Decreased critical swimming velocities (U_{crit}) at the parr-smolt transformation has been documented for coho salmon (*Oncorhynchus kisutch*, Flagg and Smith 1982) and suspected for California chinook salmon (*O. tshawytscha*) from the American River (D.T. Castleberry, J.J. Cech, Jr., M.K. Saiki, and B.A. Martin, unpublished data). These decreases may reflect structural/functional changes in swimming muscle, signalling the optimal time for migration. Increased U_{crit} has been documented for both cohos and sockeye salmon (*O. nerka*), when exposed to increased temperatures (Griffiths and Alderdice 1972, Brett and Glass 1973). As part of a larger study to quantitatively evaluate important water quality

variables (water temperature and flow) on muscle and swimming functions in developing young chinook salmon, we measured the swimming performance of Mokelumne River fish at two temperatures. In accordance with literature results, we hypothesized that these juvenile chinook salmon would demonstrate a decreased U_{crit} after reaching smolting size and an increased U_{crit} at increased temperatures.

Methods

Juvenile chinook salmon parr and smolts were collected using a minnow seine and screw traps from the Mokelumne River during the April - May, 1998, stream rearing and smoltification period. Fish were rapidly transported back to the UC Davis laboratory and held in 1.3 m-diameter insulated fiberglass tanks at the collection temperature (12ºC) for swimming performance measurements. Holding temperatures for half of the fish were increased to 17ºC over the 4-d capture-recovery period. U_{crit} (cm s^{-1}) was determined on individual chinook salmon using a recirculating swimming flume at their respective holding temperature and standard techniques (Brett 1964, Beamish 1978).

Results and Discussion

Preliminary results indicate that the Mokelumne chinook salmon demonstrate a relatively linear positive change in U_{crit} with increasing standard length and body mass (Table 1). These data do not appear to support our hypothesis that the Mokelumne River chinook salmon decrease their swimming performance at the standard lengths associated with smoltification (>60 mm). These differences may be due to species or home-stream differences in juvenile salmon. Because the Mokelumne River flows (and, consequently, river temperatures) are regulated by a number of dams and agricultural diversions upstream from the chinook parr-smolt habitat, it is also possible that regulated flows, along with 1998 El Nino climate conditions, are impacting the muscular and swimming performance development of the juvenile salmonids, creating a disparity between our data and those previously collected. Identical studies on the American River chinook are currently in progress. It is expected that these additional data will better elucidate possible differences between fall-run Mokelumne River and American River chinook salmon.

Table 1. Swimming performance (partial data set) for Mokelumne River juvenile chinook salmon at 12 and 17ºC. U_{crit} and mass are presented as means for the sample size (n) of each length class.

Standard Lengths (mm)	Temp. (C)	Ucrit (cm/sec)	Mass (g)
38-51 (n= 4)	12	26.5	1.0
58-68 (n= 5)	12	39.9	2.8
71-79 (n= 5)	12	43.5	4.6
85 (n= 1)	12	51.5	8.4
35-51 (n= 4)	17	31.5	1.1
65-76 (n= 2)	17	37.7	3.7
72-80 (n= 5)	17	42.7	5.4
85-87 (n= 2)	17	49.2	6.6

Additionally, there does not appear to be a difference in the critical swimming speed between juvenile chinook salmon at the two acclimation temperatures (Table 1). These results conflict with the temperature/swimming performance trends in the literature wherein several studies have demonstrated a significant increase in salmonid swimming performance with a rise in temperature (Brett and Glass 1973, Griffiths and Alderdice 1972). Results from our experiments are preliminary and the sample sizes are small. Further experiments will allow for more quantitative temperature comparisons of U_{crit}.

Literature Cited

Beamish, F.W.H. 1978. Swimming capacity. In: W.S. Hoar and D.J. Randall (eds.) Fish Physiology, Vol. 7 (Locomotion). Academic Press, New York.

Brett, J.R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Bd. Can. 21:1183-1226.

Brett, J.R. and N.R. Glass. 1973. Metabolic rates and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Can. 30:379-387.

Flagg, T.A. and L.S. Smith. 1982. Changes in swimming behavior and stamina during smolting in coho salmon. In: E.L. Brannon, (ed.) Proc. Symp. Salmonid Migration. Seattle, June, 1981.

Griffiths, J.S. and D.F. Alderdice. 1972. Effects of acclimation and acute temperature experience on the swimming speed of juvenile coho salmon. J. Fish. Res. Bd. Can. 29:251-264.

National Research Council (NRC) 1996. Upstream: salmon and society in the Pacific Northwest. Nat. Acad. Press. Washington.

ENERGETICS OF SWIMMING AND FEEDING

IN LARVAL FISH

I. Hunt von Herbing.
School of Marine Sciences, University of Maine, Orono, ME
Ph: (207) - 581- 4362, FAX: (207) – 581 – 4388, email:
ivonherb@umenfa.maine.edu

EXTENDED ABSTRACT ONLY DO NOT CITE

Marine fish undergo extremely high rates of mortality between the egg and juvenile stages. Survival of larval fish in the ocean has been reported to be less than 1% (Hewitt et al. 1985). The majority of this mortality occurs in the first few weeks after hatching when yolk-sac larvae shift from endogenous to exogenous feeding. This transition is associated with the morphologically and functionally underdeveloped state of a fish larva and the high energetic cost associated with searching for and capturing a prey item (Hunt von Herbing and Boutilier, 1996, Hunt von Herbing et al. 1996a, b).

In this study, costs of foraging were estimated for Atlantic cod larvae from the first-feeding stage (stage 1), when the larva was still partially reliant on yolk for energy, to the exogenous feeding stage (stage 3), when the larva depends on external prey. Foraging patterns influence encounter rates between the predator and the prey and influence the energetic benefit: cost ratio of feeding. In turn, encounter influences the probability of capture. However, encounter does not guarantee success at capture. In developing larval fish, feeding success and the energetic cost of foraging is influenced by changes in morphology and function that occur throughout ontogeny. In this study I have attempted to link changes in development in larval cod to foraging patterns, the cost of foraging and their importance to encounter, pursuit and capture of a prey item.

Values for the metabolic costs of foraging have been difficult to determine in larval fish because they generally swim at low to intermediate Re numbers ($10<Re<200$) and cannot swim at constant speeds against water flows in swim-tunnels. In this study, the cost of swimming during foraging was determined in

larval Atlantic cod (*Gadus morhua)*. Larval cod fed on two different prey types; slow swimming protozoans and rapid swimming copepod nauplii. Three-dimensional foraging patterns were determined using two orthogonal video cameras and an image analysis system. Digitization of the position of the head, tail of the predator and the prey provided data for calculation of three-dimensional swimming speeds during foraging. Metabolic rate measurements were obtained from specialized larval swimming respirometers and additional video. Together these results provided values for the costs of swimming during foraging from the first-feeding to exogenous feeding stage in larval Atlantic cod (Table 1).

Table 1. Variables and metabolic cost of foraging in larval cod feeding on protozoans.

Variable		Fish Size Class		
		1	2	3
Burst	Duration (s)	0.99 ± 0.08	0.88 ± 0.12	0.46 ± 0.13
	Distance (cm)	0.43 ± 0.08	0.33 ± 0.11	0.23 ± 0.13
	Speed(cm/s)	0.57 ± 0.06	0.56 ± 0.09	0.94 ± 0.10
Specific O_2 Uptake (mgO_2 gdwt h^{-1})		35.33	34.75	56.82
Glide	Duration(s)	1.41 ± 0.17	1.23 ± 0.27	0.92 ± 0.30
	Distance(cm)	0.17 ± 0.02	0.19 ± 0.03	0.08 ± 0.03
	Speed(cm/s)	0.16 ± 0.01	0.17 ± 0.02	0.15 ± 0.02
Specific O_2 Uptake (mgO_2 gdwt h^{-1})		2.21	2.21	4.52
Attack	Duration(s)	0.29 ± 0.07	0.33 ± 0.09	0.46 ± 0.13
	Distance(cm)	0.12 ± 0.02	0.12 ± 0.03	0.18 ± 0. 04
	Speed(cm/s)	0.99 ± 0.21	1.32 ± 0.25	1.59 ± 0.39
Specific O_2 Uptake (mgO_2gdwt h^{-1})		59.73	78.902	94.59

Each foraging event was partitioned into five major components; search, perception, pursuit, attack and capture. Larval fish searched and pursued their prey using a burst-and-glide swimming pattern. The energetic cost of both the burst and glide variables was assessed for all components of each foraging event

for the 3 developmental stages of larval cod. Using a model that included changes in swimming speed and metabolic expenditure for each component of a foraging event, costs of foraging were obtained for larval cod as they progressed from first-feeding to obligate exogenous feeding. Burst and attack speeds increased while glide speeds did not change significantly with growth (Table 1). For both burst and attack variables specific oxygen uptake increased with growth. Therefore, collectively the costs of swimming and foraging increased with growth and development as the larva progressed from endogenous and exogenous food sources. Protozoans were the preferred food and easily captured by first-feeding larvae, but only provided a net energy gain in the first and second weeks after hatching (Table 2). Larger larval size and higher swimming speeds facilitated a shift in preferred food from a prey item that was easier to capture but of lower energetic value (protozoans), to a more elusive prey item of

Table 2: Energy expenditure and net energy gain of larval cod foraging on protozoans.

Small Prey (e.g. Protozoans)

Fish Size	Prey Energy Content (E_I) (mJ)	Total Energy Expenditure (E_E) (mJ)	Total Energy Cost (E_o) (mJ)	Total Pursuit Time (T_t) (s)	Net Energy Gain (NEG) (mW)
1	0.3	0.036	2.35	4.78	0.055
2	0.3	0.201	4.64	5.79	0.017
3	0.3	0.810	13.96	3.11	-0.161

higher energy content (nauplii). This suggests that larval fish must increase their prey capture efficiency with growth in order to offset the increasing higher costs of swimming during foraging.

Acknowledgements

Many thanks to Scott Gallager and Woods Hole Oceanographic Institution for technical and soft-ware support. This project was funded from US GLOBEC NSF/OCE 9313680

References

Hewitt R.P. , Theilacker, G.H. and Lo L.C. 1985. Causes of mortality in young jack mackeral. Mar. Ecol. Prog. Ser. 26:1-10.

Hunt von Herbing, I. and R.G. Boutilier. (1996) Activity and metabolism of larval Atlantic cod (*Gadus morhua*) from Scotian Shelf and Newfoundland source populations Marine Biology, Vol. 226:607-617.

Hunt von Herbing, I. T. Miyake, B.K. Hall and R.G. Boutilier. (1996a). The ontogeny of feeding and respiration in larval Atlantic cod, *Gadus morhua*, Teleostei, Gadiformes : (I) Morphology. Journal of Morphology, Vol. 227:15-36

Hunt von Herbing, I. T. Miyake and B.K. Hall. (1996b) The ontogeny of feeding and respiration in larval Atlantic cod (*Gadus morhua*): (II) Function. Journal of Morphology, Vol. 227:37-50

TAIL KINEMATICS OF *SCOMBER JAPONICUS*: DO MACKERELS PRODUCE DORSOVENTRALLY ASYMMETRICAL TAIL MOVEMENTS?

Gibb, A. C.
California State University, Fullerton, Department of Biological Science
P. O. Box 6850, Fullerton, CA 92834-6850 USA
Phone: (714) 278-4236, FAX: (714) 278-3426, Email: agibb@fullerton.edu

Dickson, K. A., California State University, Fullerton

Lauder, G. V., University of California, Irvine

Introduction

Teleost fishes typically possess a homocercal caudal fin with a symmetrical morphology in which the dorsal and ventral lobes of the tail fin are the same size and project posteriorly beyond the axis of the vertebral column. In contrast, many elasmobranchs and some primitive actinopterygian fishes possess a heterocercal caudal fin with an asymmetrical morphology in which the ventral lobe of the fin is smaller than the dorsal lobe and the vertebral column extends upwards into the dorsal lobe of the fin. Early work with tail models (Affleck, 1950, Grove and Newell, 1936) demonstrated that a mechanical heterocercal tail will produce an upward directed force on the tail when the tail is oscillated laterally, whereas a mechanical homoceral tail produces neither an upward nor a downward directed force.

These experiments have led to the common assumption that the homocercal tail produces no significant vertical forces during steady swimming. In addition, fishes in the family Scombridae represent an extreme example of homocercal tail morphology among the teleosts: they possess semi-lunate or lunate tails that are extremely dorsoventrally symmetrical, very inflexible, and have reduced intrinsic tail musculature (Fierstine and Walters, 1968). Thus, scombrids are an ideal group to use to test assumptions about the function of the homocercal tail during steady swimming.

Methods

We examined the tail kinematics of the chub mackerel, *Scomber japonicus*, during steady swimming at several speeds [1.2, 2.2, and 3.0 total lengths per second (TL/s)]. Before the swimming trials, fish were anesthetized and small black markers were placed on the caudal fin to identify specific regions of the tail. After recovery from the anesthesia, fish swam in a flow tank at the target speeds, and were videotaped from the lateral and posterior views simultaneously. Video images were digitized, and the displacements of points on the tail fin were calculated in all three dimensions. From these data, the amplitude and timing of movements of specific tail regions were determined for four tail beats from each individual at each speed. Finally, preserved specimens of *Scomber japonicus* were dissected to describe the morphology of the tail and to identify intrinsic tail muscles.

Results

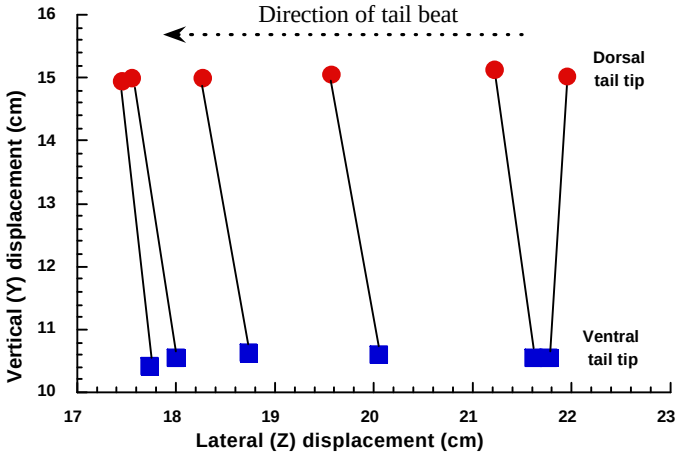


Figure 1. One half-beat illustrating the position of the tail tips at six sequential points in time. Note that the tail tips have been connected by a line to subtend the angle made by the fin.

Results presented here focus on the dorsal-ventral (Y dimension) and medial-lateral (Z dimension) movements during the beat. Analysis of the medial-lateral tail movements reveals that the dorsal lobe of the tail undergoes an approximately 10% greater lateral excursion than does the ventral lobe at all swimming speeds.

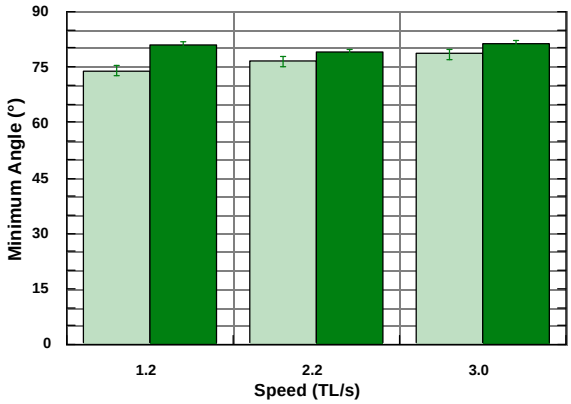


Figure 2. The average minimum angle subtended by the tail during each beat relative to its direction of travel at all three swimming speeds as determined using points on the caudal peduncle (hatched bars) and points on the tail tips (solid bars).

In addition, the dorsal lobe of the tail always leads the lower lobe of the tail during the fin beat, creating an acute angle relative to the direction of movement of the tail (Fig. 1). This angle was approximately 80° for all swimming speeds at the tail tip (Fig. 2). However, the angle was consistently more acute at the caudal peduncle (where the fin rays articulate with the vertebral column). At the caudal peduncle the angle was approximately 75° at the lowest swimming speed, and increased slightly at higher swimming speeds.

Tail dissections confirmed that *S. japonicus* have highly reduced intrinsic tail musculature. However, individuals of this species do possess two intrinsic tail muscles: a well-developed interradialis muscle and a small hypochordal longitudinalis (HL) muscle. The HL originates on the hypural plate and inserts on the base of the dorsal fin rays that comprise the tail tip. Thus, the HL is positioned such that contraction of this muscle could potentially produce dorsoventral asymmetry in the tail during the tail beat.

Conclusions

Surprisingly, the tail of *S. japonicus* does not demonstrate the type of motion predicted by the homocercal model. Instead, the dorsal lobe of the tail leads the lower lobe during each beat, creating an acute angle relative to the direction of tail movement. This movement pattern suggests that water is pushed ventrally during the beat, creating an upward force on the tail. Recent work on the homocercal tail of the bluegill, *Lepomis macrochirus*, has demonstrated that tail kinematics in this species also do not fit the homocercal tail model, and that the dorsoventrally asymmetrical tail movements in *L. macrochirus* may be generated *via* the hypochordal longitudinalis muscle (Lauder, 1995, Lauder, 1997). However, asymmetrical movements in *S. japonicus* are also present in the caudal peduncle, which is anterior to the hypochordal longitudinalis. This suggests that dorsoventral asymmetry in *S. japonicus* is generated by the axial musculature and transmitted to the caudal fin.

References

Affleck, R. J. 1950. Some points in the function, development and evolution of the tail in fishes. Proc. Zool. Soc., Lond. 120: 349-368.

Fierstine, H. L. and V. Walters. 1968. Studies in locomotion and anatomy of scombrid fishes. Mem. S. Calif. Acad. Sci. 6: 1-31.

Grove, A. J. and G. E. Newell. 1936. A mechanical investigation into the effectual action of the caudal fin of some aquatic chordates. Ann. Mag. Nat. Hist. 11: 280-290.

Lauder, G. V. 1995. Caudal fin locomotion by teleost fishes: function of the homocercal tail. Amer. Zool. 34: 13A.

Lauder, G. V. 1997. Function of the teleost fish caudal fin: Is asymmetrical movement generated actively or passively? Amer. Zool. 37: 75A.

SWIMMING BEHAVIOR OF SPLITTAIL

IN MULTI-VECTOR FLOW REGIMES:

APPLICATIONS FOR FISH SCREENS

Joseph J. Cech, Jr.
Department of Wildlife, Fish, and Conservation Biology
University of California
Davis, CA 95616
(530) 752-3103, FAX (530) 752-4154,
email: jjcech@ucdavis.edu

Christina Swanson[1] and Paciencia S. Young[2]
Department of Wildlife, Fish, and Conservation Biology
University of California, Davis, CA 95616
[1](530) 752-8659, FAX (530) 752-4154, email: cswanson@ucdavis.edu
[2](530) 754-4398, FAX (530) 752-4154, email: psyoung@ucdavis.edu

Introduction

The splittail, *Pogonichthys macrolepidotus* (Ayres), used to be one of the most abundant estuarine species in the Sacramento-San Joaquin estuary to which it is endemic and supported a small, but enthusiastic, hook-and-line fishery. It is considered primitive compared to other endemic cyprinid fishes of the California central valley because of its two rows of pharyngeal teeth in contrast to the more advanced characteristic of one row (Caywood 1974, Moyle 1976). It was once widely distributed throughout the California central valley but disappeared from much of its native range because of loss or alteration of lowland habitats following dam construction, water diversion, and agricultural development, and is now restricted to the estuary (Herbold et al. 1992). Juvenile splittail are now subject to entrainment and impingement at >2000 water diversions located within their current habitat.

Three alternatives currently being considered to improve estuarine habitats include construction of large fish screens to better separate fish from water diverted for municipal and industrial (including agricultural) uses. Water in front of these large screens would have complex flows from both approach

(diversion pump induced, through the screen) flows and sweeping (river or tidal-induced, parallel to the screen) flows. Our objective was to examine the swimming performance and behavior of splittail exposed to complex flow regimes in a circular flume, equipped with a fish screen, termed a “fish treadmill."

Methods

Splittail swimming performance was measured using screen contact rates (including impingements lasting $\geq$5 min) and post-experimental survival rates, whereas their swimming velocity, swimming gaits, and rheotaxis behavior was measured using video tapes and a computerized motion analysis system (Peak Performance). Water velocities were measured at >100 locations within the swimming channel using an acoustic doppler velocimeter (SonTek) and detailed flow velocity maps were generated for each experimental flow regime. Twenty fish per experiment were exposed to 10 combinations of approach (0, 6, 10, 15 cm/s) and sweeping (0, 31, 62 cm/s) flows in the fish treadmill's 60 cm-wide swimming channel, and screen contacts and impingements were continuously recorded. Post-experimental mortalities were recorded after 48 h. Videotaped tracks of 5 fish exposed to 2 combinations of approach (0, 15 cm/s) and sweeping (0, 62 cm/s) flows were analyzed at 10 different times throughout the 2-h experiment for behavioral measurements. The fish treadmill apparatus offers a useful alternative to the single-vector "Brett-type" flume used by many researchers and agencies in developing fish screen velocity criteria.

Results and Discussion

Preliminary results from experiments at 10 flow combinations are available for swimming performance (Table 1) and at 2 flow combinations (0 cm/s approach with 0 cm/s sweeping flows combination [0/0], and 6/62) for swimming behavior. No splittail were impinged on the fish screen, and no splittail died during the experiments or during the subsequent 48-h periods (Table 1). They swam closer to the screen (mean$\pm$SE: 20.4$\pm$3.4 cm) and contacted it more often (2.6$\pm$0.3 contacts/[min*fish]) at the 0/0 conditions, compared with the 6/62 conditions (53.7$\pm$0.8 cm, 0 contacts). Their contacts, under these control (non-flowing) conditions, were of the "exploratory" nature, rather than forced. Splittail also swam closer to the surface (22.8$\pm$3.9 cm from the bottom) at 0/0, compared with 8.2$\pm$0.9 cm at 6/62 conditions. Splittail swimming velocity did

not appear to be related to flow regime (43.0±2.8 cm/s at 0/0, 49.8±4.5 cm/s at 6/62). Interestingly, these swimming velocities were 10-15 cm/s higher than the critical swimming velocities measured previously for splittail of this size at similar temperatures (Young and Cech 1996). Possible reasons for the faster velocities in the fish treadmill include possible reduced-activity ("rest") periods between fast swimming periods, hydrodynamic advantages from following others in a school, or the lack of relative confinement inherent in the single-vector flume.

Flow		Size	Screen Contacts	Impinged	Mortality	
appr. (cm/s)	sweep.	(cm SL) (mean±SE)	(contacts/fish*min) (mean±SE)	(%)	0 h (%)	48 h
0	0	5.1±0.6	2.65±0.33	0	0	0
6	0	5.5±0.1	0.39±0.05	0	0	0
10	0	5.4±0.1	0.01±0.01	0	0	0
15	0	5.5±0.1	0.35±0.05	0	0	0
6	31	4.7±0.1	0.00±0.00	0	0	0
10	31	4.4±0.1	0.00±0.00	0	0	0
15	31	5.2±0.1	0.08±0.02	0	0	0
6	62	5.4±0.1	0.00±0.00	0	0	0
10	62	5.4±0.1	0.06±0.03	0	0	0
15	62	5.6±0.1	0.02±0.02	0	0	0

Table 1. Fish standard length (SL), screen contacts, impingement percentage, and mortality rates of splittail exposed to ten multi-vector flow regimes in the fish treadmill at 19°C.

Results of these and ongoing studies will provide detailed and more realistic information on how splittail respond to multi-vector flow regimes and fish screens. This information will help environmental managers to develop fish screen flow criteria that minimize harm to this species.

Acknowledgment

This research was supported by the California Department of Water Resources. We thank M. L. Kavvas, Z. Chen, E. Velagic, H. Bandeh, A. Karakas, E. Dogrul, W. Summer, D. Barnum, and L. Kaelin, Hydraulics Laboratory, Department of Civil and Environmental Engineering, UC Davis; G. Edwards, D.

Killam, R. Soto, P. Macias, D. Domich, and J. Osmondson, California Department of Fish and Game; R. Brown, D. Hayes, T. Frink, S. Mayr, D. Shigematsu, California Department of Water Resources; and M. Danley, K. Day, N. Hutt, S. Lema, M. Lee, D. Warren, M. Gonzalez, B. Begun, M. Karagosian, R. Mayfield, S. Katzman, Department of Wildlife, Fish, and Conservation Biology, UC Davis, for their assistance with this project.

References

Caywood, M.L. 1974. Contributions to the life history of the splittail *Pogonichthys macrolepidotus* (Ayres). M.S. Thesis, California State University, Sacramento, 78 pp.

Herbold, B., A.D. Jassby, and P.B. Moyle. 1992. Status and trends report on aquatic resources in the San Francisco Estuary. Public Report of the San Francisco Estuary Project. 257 pp.

Moyle, P.B. 1976. Inland fishes of California. Univ. Calif. Press. Berkeley, 405 pp.

Young, P.S. and J.J. Cech, Jr. 1996. Environmental tolerances and requirements of splittail. Trans. Am. Fish. Soc. 125:664-678.

SWIMMING BEHAVIOR OF DELTA SMELT

IN MULTI-VECTOR FLOW REGIMES:

APPLICATIONS FOR FISH SCREENS

Christina Swanson
Department of Wildlife, Fish, and Conservation Biology
University of California
Davis, CA 95616
(530) 752-8659, FAX (530) 752-4154, email: cswanson@ucdavis.edu

Paciencia S. Young[1] and Joseph J. Cech, Jr.[2]
Department of Wildlife, Fish, and Conservation Biology
University of California
Davis, CA 95616
[1](530) 754-4398, FAX (530) 752-4154, email: psyoung@ucdavis.edu
[2](530) 752-3103, FAX (530) 752-4154, email: jjcech@ucdavis.edu

Introduction

The delta smelt, *Hypomesus transpacificus*, is a small osmerid found only in California's Sacramento-San Joaquin estuary (Moyle et al., 1992). Following a drastic population decline in the early 1980s and continuing low population levels, the species was listed as threatened under both federal and state Endangered Species Acts. Entrainment and impingement losses of larval, juvenile, and adult fish at the more than 2200 screened and unscreened water diversions located within its habitat have contributed to the delta smelt's decline (Moyle et al., 1992). Detailed investigations of delta smelt swimming in unidirectional flows in a Brett-type flume indicated that the fish are moderate swimmers with distinct velocity-dependent swimming behaviors and both behavioral and kinematic limitations on swimming at submaximal velocities (Swanson et al., 1998). Given the importance of the behavioral component in delta smelt swimming, application of results of this type of study to develop safe flow criteria for screened water diversions was problematic and might seriously misinterpret the fish's true performance in flowing waters near fish screens. The objective of this study was to examine delta smelt swimming behavior and

performance in multi-vector flows similar to those that occur near screened water diversions.

Methods

We measured aspects of delta smelt swimming behavior and performance in multi-vector flows in a large annular flume (swimming channel diameter: 4.3 m; width: 0.6 m; depth: 0.3-0.4 m) equipped with a fish screen ("fish treadmill") at 190C. In each 2-h experiment, 20 fish were exposed to an approach flow through the fish screen (0, 6, 10 and 15 cm/s) in combination with a sweeping flow past the screen (0, 31, and 62 cm/s). Flow velocities were measured at >100 locations within the swimming channel using an acoustic doppler velocimeter (SonTek) and detailed flow velocity maps generated for each experimental flow regime. In each experiment, measurements were made on screen contacts by the fish, impingement (prolonged contact with screen, >5 min), location within the swimming channel (distance from screen in cm, and depth in cm from bottom), swimming velocity (cm/s, through the water), and mortality. Screen contacts and impingement were measured visually throughout the experiment. Fish position within the channel and velocity were measured from video tape records using a computer-assisted, video capture/motion analysis system (Peak Performance Technologies, Inc., Englewood, CO) on five fish at 10 selected times during the experiment. Mortality was measured at the end of the 2-h experiment and 48-h post-experiment. Fish size (wet weight in g, and standard length in cm) was measured at the time of death or the end of the 48-h post-experiment period.

Results and Discussion

Delta smelt performance in the complex flow field near the fish screen, as indicated by screen contact rates, impingement and survival, was most closely related to the approach flow component (Table 1). In all flow combinations except the control (0 cm/s), delta smelt experienced frequent contacts with the fish screen. In flow regimes with 0 and 31 cm/s sweeping flows, screen contact rates appeared to increase during the second hour of the experiments. In the 62 cm/s sweeping flows, screen contact rates were highest during the first 10-30 min and then generally decreased to levels comparable with rates measured in the lower sweeping flow regimes. Regardless of sweeping flow velocity, some delta smelt became impinged in approach flow regimes with >6 cm/s and these

impinged fish rarely survived the experiment. Mortality (48-h post-experiment) was related to the approach flow velocity and screen contact rates; delta smelt which experienced higher screen contact rates had higher mortality in the high approach velocity flow regimes.

Table 1. Fish size, and screen contact, impingement, and mortality rates of delta smelt exposed to multi-vector flow regimes in the fish treadmill at 190C.

Flow		Size	Screen Contacts	Impinged	Mortality	
appr. (cm/s)	sweep. (cm/s)	(cm SL) (mean±SE)	(contacts/fish*min) (mean±SE)	(%)	0h (%)	48h (%)
0	0	4.7±0.2	0	0	0	0
6	0	4.7±0.2	0.14±0.	0	0	0
10	0	5.2±0.1	0.30±0.26	5	5	35
15	0	5.2±0.2	0.09±0.19	10	10	10
6	31	4.8±0.3	0.08±0.10	0	0	32
10	31	5.2±0.3	0.01±0.02	0	0	0
15	31	5.3±0.3	0.39±0.36	15	0	10
6	62	4.8±0.4	0.11±0.10	0	0	5
10	62	4.8±0.3	0.11±0.17	5	10	15
15	62	4.4±0.2	0.54±0.54	50	60	70

In contrast to the performance of the fish in relation to the fish screen (Table 1), delta smelt swimming behavior in multi-vector flows was largely dependent on sweeping flow velocities (Table 2). Mean swimming velocity increased with flow velocity. In the high sweeping flow regimes, mean swimming velocities were comparable to critical swimming velocities (27 cm/s) reported in Swanson et al. (1998). During the 2-hour experiment, some fish appeared to fatigue and became impinged, unable to escape the screen. Position within the swimming channel, distance from the screen and depth, was also related to sweeping velocity; delta smelt swam further from the screen and closer to the bottom in the higher sweeping flows. Fish in these experiments were probably seeking a velocity refuge; the velocity of the sweeping flow was lower in areas further from the screen and closer to the bottom.

Table 2. Location, depth, and swimming velocity of delta smelt exposed to multi-vector flow regimes in the fish treadmill at 190C.

Flow velocity appr. (cm/s)	sweep.	Size (cm SL) (mean±SE)	Location (cm from screen) (mean±SE)	Depth (cm from bottom) (mean±SE)	Swimming (cm/s) (mean±SE)
0	0	4.7±0.2	24.4±1.4	13.4±1.6	15.5±2.4
6	0	4.7±0.2	17.7±1.8	28.3±4.0	21.0±2.6
10	0	5.2±0.1	19.1±2.3	18.3±2.4	20.3±2.1
15	0	5.2±0.2	22.1±2.6	24.7±3.2	24.1±2.6
6	31	4.8±0.3	29.8±2.8	10.3±1.2	15.2±1.6
10	31	5.2±0.3	29.1±1.7	11.9±1.5	27.5±2.3
15	31	5.3±0.3	21.3±2.1	14.2±2.5	25.4±2.0
6	62	4.8±0.4	44.8±1.3	10.2±2.0	31.2±2.5
10	62	4.8±0.3	36.4±1.5	5.2±0.4	30.9±1.4
15	62	4.4±0.2	30.2±2.2	11.6±0.9	25.0±1.1

Results of these and ongoing studies will provide detailed and more realistic information on how delta smelt respond to multi-vector flow regimes and fish screens. This information will help environmental managers to develop fish screen flow criteria that minimize harm to this threatened fish.

Acknowledgment

This research was supported by the California Department of Water Resources. We thank M. L. Kavvas, Z. Chen, E. Velagic, H. Bandeh, A. Karakas, E. Dogrul, W. Summer, D. Barnum, and L. Kaelin, Hydraulics Laboratory, Department of Civil and Environmental Engineering, UC Davis; G. Edwards, D. Killam, R. Soto, P. Macias, D. Domich, and J. Osmondson, California Department of Fish and Game; R. Brown, D. Hayes, T. Frink, S. Mayr, D. Shigematsu, California Department of Water Resources; and M. Danley, K. Day, N. Hutt, S. Lema, M. Lee, D. Warren, M. Gonzalez, B. Begun, M.

Karagosian, R. Mayfield, S. Katzman, Department of Wildlife, Fish, and Conservation Biology, UC Davis, for their assistance with this project.

References

Moyle, P. B., B. Herbold, D. E. Stevens, and L. W. Miller. 1992. Life history and status of the delta smelt in the Sacramento-San Joaquin Estuary, California. Trans. Am. Fish. Soc. 121:67-77.

Swanson, C., P. S Young, and J. J. Cech, Jr. 1998. Swimming performance of delta smelt: maximal performance, and behavioral and kinematic limitations on swimming at submaximal velocities. J. Exp. Biol. 201:333-345.

A PRELIMINARY APPRAISAL OF EMO TELEMETRY FOR COMPARING ACTIVITY PATTERNS OF ADULT MIGRATORY AND PRE-MIGRATORY MASU SALMON

Jill B.K. Leonard
Toya Lake Station for Environmental Biology,
Faculty of Fisheries, Hokaido University,
Abuta, Hokaido, Japan 049-57
(v) 011-81-142-75-2651 (f) 011-81-142-75-2943
Email jleonard@ccmsl .hucc.hokudai.acjp

Hiroshi Ueda
Toya Lake Station for Environmental Biology,
Faculty of Fisheries, Hokaido University,
Abuta, Hokaido, Japan 049-57

Introduction

Energy expenditure by anadromous fish is thought to be a driving force behind the evolution of migration. While energy use has been extensively studied in anadromous salmonids *(e.g.* Jorisson *et al*, 1997), there is little information comparing the energy consumed during migration to levels typical of the pre-migratory period. In an effort to make this comparison, we are using physiological telemetry with a population of lacustrine salmonids where the lake serves as a model 'ocean'. EMO telemetry has been shown to be useful in evaluating the energetic costs of fish activity in the wild (Demers *et al,*1996; Weatherley *et al,* 1982) including during fish migration through river sections @inch *et al,* 1996).

Methods

Working in a caldera lake in northern Japan, we have tagged adult, landlocked masu salmon *(Oncorhynchus masou;* 10 $fish_3$ 1.8 t 0.2 kg, 54.1 1 1.4 cm TL) with electromyographic @MG) radiotransmitters (Lotek Engineering, Inc. Newmarket, ONT, for details see Kasebo *et at,* 1992). Fish were captured by gill net, implanted with the tag, calibrated individually using a mobile flow chamber and released into the lake in September, 1997. During surgical tag implantation, fish were anesthetized with ethyl m-aminobenzoate methanesulfonate (8Omgl) and provided with artificial respiration. Tags were inserted ventrally into the peritoneal cavity and the electrodes were threaded beneath the left lateral skin so that the steel electrode tips were in the red muscle. Electrodes were configured so that the tips rested approximately 4mm apart and shielding prevented tip contact.

Fish were allowed to recover overnight and then subjected to individual calibration the following day. Fish were calibrated in a portable flow chamber attached to a small boat so that the fish was required to swim at boat speed to remain in a central position in the chamber. Fish were swum at approximately 0, 1, 2 and 3 body lengths/second for 15 rninutes at each speed to generate an individual calibration relationship between EMG pulse frequency and swimming speed. Typically, calibration resulted in a significant, negative linear relationship between swimming speed and pulse frequency with ?>.90. Following calibration, fish were released into Lake Toya, Japan. Fish were tracked both in the lake and in streams during their spawning migration. Within the lake, our protocol was to locate a tagged fish and obtain a 30 minute bout of continuous data as often as possible. We attempted to gather data from four different temporal periods: dawn, day, dusk, night. The majority of stream data was generated by an automated data acquisition station located in the largest stream flowing into Lake Toya (Sobetsu R.). Data was taken in bouts of 27 continuous pulse intervals from each individual within the stream.

Often fish implanted, some data was obtained from seven fish. In the case of three individuals, extensive data was obtained and some initial analysis is possible. We intend to increase this data set during the next spawning season. Preliminary results indicate that there is typically a significant difference in an individual's activity pattern between lake and stream based on EMG data. In the case of Fish #7 (male, 2.0 kg, 57 cm; Figure 1), activity in the stream during passage, nest defense and spawning was higher than in the lake. At other times, however, there was no difference between lake

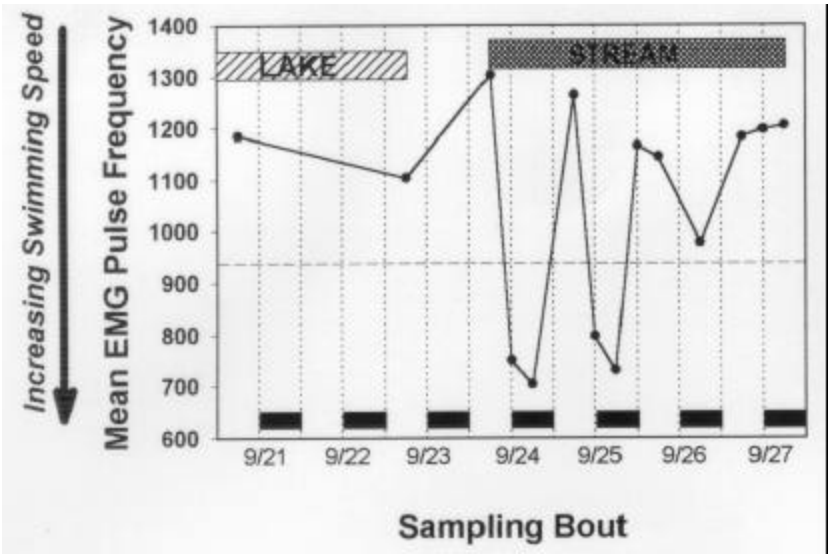


Figure 1. Mean EMG pulse frequency *(+1-* S.F.) for Fish #7 (male, 2.0kg, 57 cm Th) showing 2 data bouts from the lake and 7 from a stream over a period of 7 dayL Each data point includes 1 data bout consisting of approx. 30 niinutes real time and approx. 1000 pulse frequency intervals. Dark bars indicate the period from dusk to daw~ Dashed line indicates the maximum calibrated EMG pulse frequency for this individual (937.6EMG = 156.9 cmls).

and stream activity. Generally, activity is higher in the streams (as much as 118%), however, this difference varies in magnitude, and occasionally in direction, with the individual. Frequently, there is a bimodal distribution of EMG activity in the streams likely resulting from a mixture of aerobic and anaerobic activity. For example, this pattern occurred in data gathered from Fish #11 (male, 1.5 kg, 55 cm TL) during rapid upstream migration in the Okawa River Figure 2). However, analysis suggests that this bimodal pattern does not occur at all times during the day and is a characteristic of certain behaviors (rapid travel upstream, spawning, nest defense) rather than of stream residency.

While more data are required to generate conclusions regarding the comparative energetic expense of stream and lake residency, we believe that EMG telemetry will be a fruitfbl method for evaluating this relationship. Preliminary results suggest that there are differences in energetic expense between these two environments, likely linked to specific behaviors, and these differences are quantifiable using this methodology.

References

Hinch, S. G., RE. Diewert, T.J. Lissimore, A.M.J. Prince, M.C. Healey and M.A. Henderson. 1996. Use of electromyogram telemetry to assess difficult passage areas for river-migrating adult sockeye salmon. Trans. Amer. Fish. Soc.. 125:253-260.

Jonsson, N., B. Jonsson and L.P. Hansen. 1997. Changes in proximate composition and estimates of energetic costs during upstream migration and spawning in Atlantic salmon, *Salmo salar*. J. Anim. Ecol. 66:425-436.

Kasebo, P. A., A.H. Weatherley, J. Lotimer and M.D. Farina. 1992. A biotelemetry system recording fish activky. J. Fish Biol. 40:165-179.

Weatherley, A. H., S.C. Rogers, D.G. Pincock and J.R. Patch. 1982. Oxygen consumption of active rainbow trout, *Salmo gairaneri* Richardson, derived from electromyograms obtained by radiotelemetry. J. Fish Biol. 20:479-489.

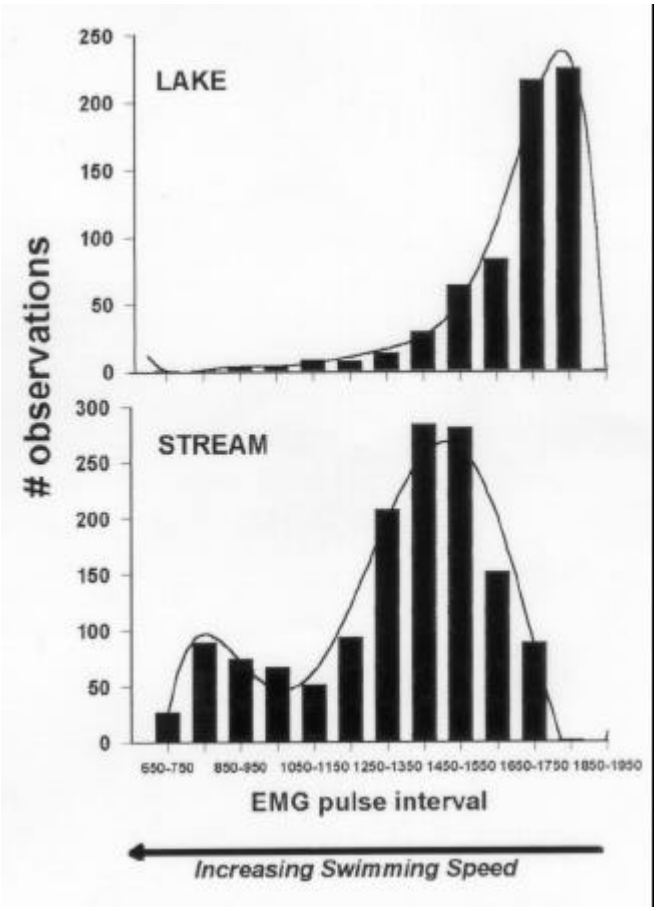


Figure 2. Frequency distribution of data from Fish #11 (male, 1.5 kg, 55 cm TL) received on 9/19197. 'Lake' is data received while fish was located at mouth of the Okawa River. 'Stream' is data following entrance of fish into Okawa IL (visually confirmed). Total data set approximately 1 hour in duration.Demers, B., R.S. McKinley, A.H. Weatherley and D.J. McQueen. 1996. Activity patterns of largemouth and smailmouth bass determined with electromyogram biotelemetry. Trans. Amer. Fish. Soc. 125:434-439

DEVELOPMENT AND APPLICATION OF SPRINT TESTS FOR ASSESSING SWIM PERFORMANCE IN JUVENILE SALMONIDS

Gordon McDonald
Department of Biology[1] McMaster University
1280 Main Street West, Hamilton, Ontario Canada L8S 4K1
email: mcdonald@mcmaster.ca

Wendy McFarlane[1] and Louise Milligan[2]
Department of Zoology[2]
University of Western Ontario

Introduction

The two most common procedures for assessing swim performance in fish, including salmonids, is the fast-start or escape response, and the critical swimming speed (U_{crit}). These tests, in essence, measure two extremes: the maximum velocity achievable and the maximum velocity sustainable. For age 0^+ rainbow trout (<10 cm body length) these limits are >13 BL s^{-1} for <200 msec (Domenici and Blake, 1997) and <7 BL s^{-1} for >200 min (Beamish, 1978). While the two extremes are well characterized, very little is known about performance limits in the 'prolonged swimming'range between them. This range is important because it encompasses a significant fraction of normal swimming behavior. Hence the objective of this study was to develop procedures for assessing 'prolonged' swimming performance. Our approach was to use the 'fixed velocity' sprint test first proposed by Brett (1967) where fish are accelerated to a constant velocity and held at that velocity until exhausted. Specifically, we were interested in the variability and reproducibility of individual sprint performance and how it scales with body size, how duration is affected by test velocity, and the effect of rate of acceleration on swim duration. We also looked at performance of fish swum in groups; our interest being to develop a large scale, rapid screening tool for assessing swim capacity. Finally

we looked at the anaerobic energy contribution to sprint activity and developed a procedure for assessing anaerobic capacity.

Methods

Most of the experiments were performed on fingerling (age 0^+) rainbow trout, ranging in size from 5-10 cm (1 to 8 g) but fingerling brook trout and Atlantic salmon of similar size were used for comparative purposes. Experiments were conducted within the temperature range of 7 -16° C.

Sprint testing was carried out in a 100 L open swim flume (Vogel, 1978 design). Fish were transferred to the flume either individually or in groups for a brief period of acclimation (usually 5 min) to an orienting flow of ~1 BL s^{-1}. Tests evaluated the following variables: group size from 1-10 fish, acceleration times ranging from 2-60 min, and final test velocities ranging from 20 to 50 cm s^{-1}. Fish were swum until exhausted; failure to resume swimming after 3 successive prods was the criterion chosen for exhaustion. In some instances, fish were terminally sampled at exhaustion for measurement of muscle or whole body metabolites. Concentrations of lactate, phosphocreatine (PCr) and ATP were used to calculate, in ATP equivalents, the anaerobic energy expenditure (AEE in $\mu M\ g^{-1}$). A fixed duration, maximum exertion test (8 min of continuous manual stimulation) was used to produce maximum AEE, i.e. anaerobic capacity.

Results

Body size and rate of acceleration were the most important determinants of sprint duration (endurance) at any test velocity. Endurance scaled exponentially to body length with an exponent in the range of 4-6. Endurance increased linearly with an increase in the duration of the acceleration phase of the sprint test from 2 to 60 min.

Individual sprint performance was highly reproducible and unaffected by whether fish were tested alone or in groups. However, there was a large inter-individual variability in performance; of which only 30% or less was explained by size variation.

Sprint duration decreased as the log of the sprint velocity. However, the residual variation in performance remained the same, even at high test velocities.

The pattern of fuel usage in muscle (glycogen, ATP and PCr) indicates that performance in the sprint test has both aerobic and anaerobic components and that exhaustion usually sets in before anaerobic utilization is complete.

Conclusions

Fixed velocity testing is a reliable, reproducible method for assessing stamina in juvenile salmonids providing correction is made for the large scaling coefficients. Furthermore, it is a practical screening tool because it can readily be adapted to test large numbers of fish over short test intervals.

Sprint performance, corrected to a reference body size, exhibits a large inter- and intra-specific variability. This variability appears to reflect true inter-individual variability in swim capacity attributable to either or both anaeobic and aerobic capacity

Juvenile salmonids recover rapidly from sprints to exhaustion because they employ only a fraction of their anaerobic resources in each sprint.

References

Beamish, F. W. H. 1978. Swimming capacity. Pages 101-187 *in* W. S. Hoar and D. J. Randall, editors. Fish Physiology. Vol.VII. Academic Press, New York.

Brett, J. R. 1967. Swimming performance of sockeye salmon (*Oncorhynchus nerka*) in relation to fatigue time and temperature. J. Fish. Res. Board Can. 24: 1731-1741.

Domenici, P., and R. W. Blake. 1997. The kinematics and performance of fish fast-start swimming. J. exp. Biol. 200: 1165-1178.

Vogel, S., and M. LaBarbera. 1978. Simple flow tanks for research and teaching. Biosciences 28: 638-643.

IMPROVING SPRINT PERFORMANCE

OF JUVENILE RAINBOW TROUT

THROUGH SELECTION AND TRAINING

Wendy J. McFarlane
Department of Biology, McMaster University
1280 Main Street West, Hamilton, Ontario Canada L8S 4K1
email: mcfarlwj@mcmaster.ca

Gordon McDonald
Department of Biology, McMaster University

Introduction

Our recent work (McDonald et al, 1998) has shown that individual swim performance of juvenile salmonids in prolonged swimming trials (i.e. sprint trials) is quite variable. For example, times to exhaustion in a fixed velocity test at 40 cm s^{-1} varied by over 20 fold (from 25 to 520 seconds) in rainbow trout ranging in size from only 4.0 to 5.8 cm fork length. Moreover, very little of the variability (typically less than 30%) can be attributed to size. Nonetheless, individual performance was highly reproducible over successive trials. This suggests that the variability is an inherent, non-random trait. In this study we have exploited the individual variability of juvenile rainbow trout to address two questions: is it possible to select trout for sprint performance? and secondly, is it possible to improve performance through sprint training? At the same time we examined the nature of the anaerobic fuel use during sprints to exhaustion to determine the basis of differences amongst individuals and whether patterns of fuel use are affected by training.

Methods

All experiments were performed on juvenile rainbow trout (1-3 g) held in circular 40 L tanks at 8-10 C, and fed a 4% ration of commercial trout feed. Fish were exercised using an open, recirculating swim flume (~100 L volume). This study employed four swimming tests; a fixed velocity (FV) sprint test to

exhaustion, a fixed duration (FD) maximum exertion test, a continuous sub-maximal exertion test and a 30 minute incremental velocity test (U_{crit}). The FV test (McDonald et al, 1998) was used to select fish based on performance and as one of three training regimes. In the FV test, fish are rapidly accelerated to ~7 BL/s and swum to exhaustion. The FD test was used as a training procedure (chase-training) and, in conjunction with measurements of glycogen, ATP, phosphocreatine and lactate, to assess maximum anaerobic energy expenditure. The FD test comprised manual individual stimulation for 8 min, a procedure shown to produce maximum exertion (McDonald et al, 1998). The third training regime was continuous exercise in the flume at 1.5 BL/s.

To select for performance, fish were sprinted to exhaustion in groups of 10, sprint duration was corrected for size and the fish were sorted into high, medium and low performance groups (High n=194; Medium n=210; Low n=197).

Sprint training was carried out by sprinting fish in groups of 10, to exhaustion, once daily for 14 days. Chase-training was carried out with the FD test, once every two days, for 14 days. For continuous aerobic exercise, 20 fish were held in a swim tunnel at 1.5 BL/s for 14 days. For analysis of metabolites, fish were terminally sampled prior to and at the end of exercise and freeze-clamped whole. White muscle was removed and ground under liquid nitrogen, with aliquots of tissue then analyzed for glycogen, lactate, ATP and phosphocreatine (PCr), according to Bergmeyer (1983).

Results and Discussion

After selection there was no overlap in sprint performance between the high and low performance groups. Their sprint durations, expressed as times to 50% exhaustion, were 282 ± 1 sec (n = 195) and 63 ± 11 sec (n = 211), respectively. Over the next 4 weeks both groups grew equally well (specific growth rate of 3.79 ± 0.21 % BW/day and 3.60 ± 0.23 % BW/day respectively), and when a subsample was retested, still showed a dramatic difference in sprint performance (High 306 ± 18 sec, n = 30; Low 117 ± 4 sec, n = 30). Ucrits measured at this time were also significantly higher in the high performance group (High 7.31 ± 0.12 BL/s, n = 20; Low 6.47 ± 0.11 BL/s, n=20). We interpret this finding as indicating that the sprint selection procedure has, in fact, selected for aerobic capacity. Indeed, there was no apparent difference in anaerobic capacity between the two groups. Upon exhaustion in the sprint trials both groups had utilized similar amounts of glycogen, PCr and ATP and produced the same

amount of lactate and yet the high performance group swam an average of 4 times longer than the low performance fish.

This is a substantially greater improvement in less time than previously reported for either chase-trained or continuously-trained salmonids (see Davison, 1997 for review). Nonetheless, there was a great degree of variability in performance improvement, with some fish improving dramatically, and others not at all, which explains the increasing variance in sprint durations with training (Fig. 1). In contrast, chased- and continuously-trained fish showed no improvement in sprint performance over time (Fig. 1).

Repeated sprinting produced a 2-3 fold increase in average sprint performance in as little as two weeks (for example see Fig. 1).

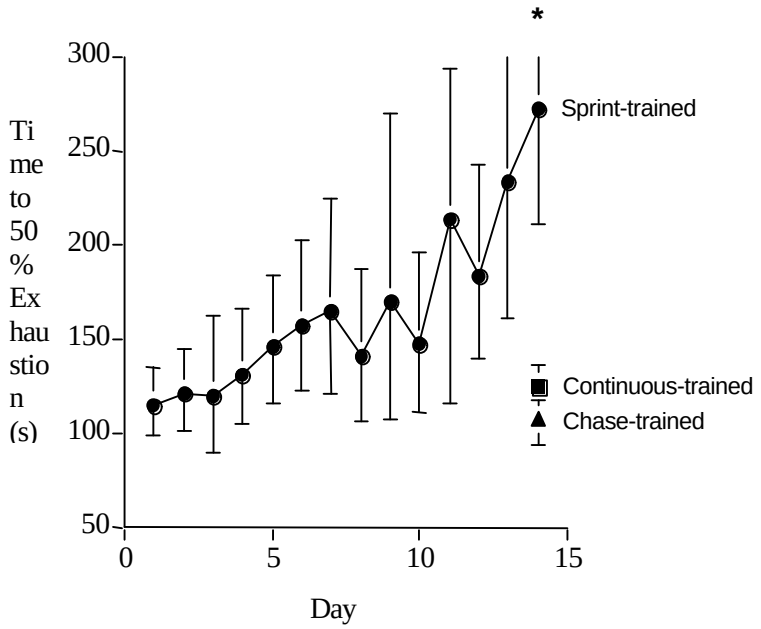


Figure 1: Sprint performance of sprint-trained chase-trained continuous-trained fish. Values are means ± 95%

Sprint-trained fish also showed substantial changes in muscle fuel use. They had higher muscle glycogen stores, utilized more glycogen during a sprint and accumulated more lactate but used less PCr and ATP than untrained fish. However, we believe that this is not a response to periodic high speed swimming

per se but rather to repeated exhaustion because the chased-trained fish developed a virtually identical pattern of fuel use but no improvement in sprint performance. Furthermore, the continuously-trained fish showed no difference in fuel use compared to untrained fish possibly because they were never exhausted by the training regime. We conclude that the fixed velocity sprint test is a powerful tool for selecting or improving sprint performance in salmonids and for providing insight into aerobic and anaerobic limits to performance.

Acknowledgements

This work was supported by an NSERC operating grant awarded to DGM, and an Ontario Graduate Scholarship awarded to WJM.

References

Bergmeyer, H.U., Ed. 1983. *Methods of Enzymatic Analysis*. Basel, Verlag Chemie.

Davison, W. 1997. The effects of exercise training on teleost fish, a review of recent literature. Comp. Biochem. Physiol. 117A: 67-75.

McDonald, D.G., W.J. McFarlane and C.L. Milligan. 1998. Anaerobic capacity and swim performance of juvenile salmonids. Can. J. Fish. Aquat. Sci. 55 (5): in press

PHOTIC REGULATION OF *IN VITRO* MELATONIN PRODUCTION FROM THE PINEALS OF TWO SPARIDS

Benny Ron
Fish Physiology Department, National Center for Mariculture, Israel
Oceanographic & Limnological Research Ltd.,
P.O. Box 1212, North Shore, Eilat 88112, Israel
phone: 972-7-6361400 / fax: 972-7-6375761 /
email: ronbenny@agri.huji.ac.il

Darren K. Okimoto
Psychology Department, University of Delaware,
Newark, Delaware, 19716, USA
phone: 01-302-831-3088 / fax: 01-302-831-3645 /
email: oki@udel.edu

Introduction

The pineal gland of fishes is a photosensitive organ that synthesizes and releases the hormone melatonin rhythmically, coincident with the ambient light:dark (LD) cycle, with low synthesis of melatonin occurring during the day and higher synthesis at night (Zachmann et al., 1992). In this paper, we present initial results from a study that investigates the properties of the melatonin-generating system in two representatives of the Family Sparidae, the gilthead seabream, *Sparus aurata*, and the double-bar seabream, *Acanthopagrus bifasciatus*.

The commercially-important *S. aurata* is a native of the Mediterranean Sea and an excellent model species since the fish is hardy, breeds readily in captivity, and can be easily maintained in high densities in the laboratory. *A. bifasciatus*, which is native to the Red Sea and the Western Pacific Ocean, has been recently domesticated, spawned, and grown in our laboratory at the National Center for Mariculture in Eilat, Israel, as a potential new species for the aquaculture industry.

Materials and Methods

The experiments were undertaken at the National Center for Mariculture in Eilat, Israel. Pineal glands were collected from post larval and juvenile *S. aurata* and juvenile *A. bifasciatus* and placed in static organ culture under 12L:12D cycle for 3-4 days at constant temperature (25°C). Culture medium was collected and renewed every 12 hr. Dark conditions was produced by wrapping each culture plate containing the fish pineals in aluminum foil. Samples were kept frozen at -20°C until melatonin determination by radioimmunoassay.

Results and Discussion

Rhythmic melatonin release under 12 hr light:12 hr dark was detected in both *S. aurata* (Fig. 1) and *A. bifasciatus* (Fig. 2), and in *S. aurata* at the post larval stage of development, with higher melatonin release occurring during the dark period.

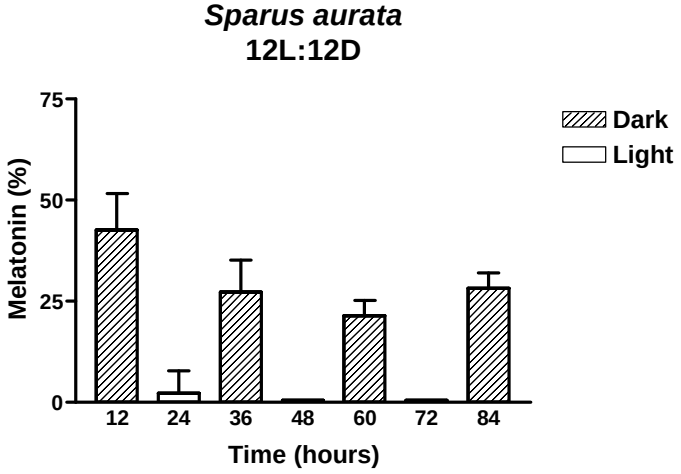


Figure 1. Under 12L:12D, melatonin release from the pineal of juvenile *S. aurata* was rhythmic, with higher melatonin released occurring during the dark than in the light.

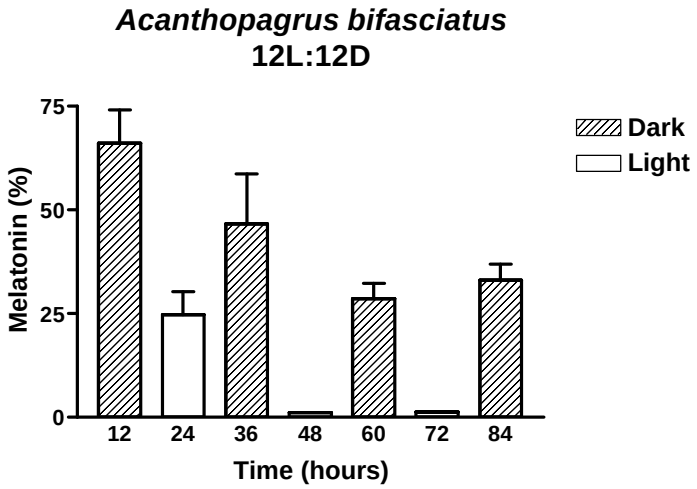


Figure 2. Under 12L:12D, melatonin release from the pineal of juvenile *A. bifasciatus* was rhythmic, with higher melatonin released occurring during the dark than in the light.

Interestingly, we observed a species difference in melatonin release during the first LD cycle, suggesting the existence of inter-species variations in the melatonin-generating system in the Sparidae family. These data suggest that the pineals of *S. aurata* and *A. bifasciatus* are photosensitive (e.g., contain photoreceptors) and that photic entrainment is present from the post-larval stage of development in *S. aurata*. Further characterization of the melatonin-generating systems of these two sparids is in progress.

References

Zachmann, A., M.A. Ali, and J. Falcon. 1992. Melatonin and its effect in fishes: an overview. In "Rhythms in Fishes" (M.A. Ali, ed.), NATO ASI series. Series A, Life Sciences, Vol. 236, pp. 149-165. Plenum Press, New York.

RHYTHMIC MELATONIN RELEASE FROM THE PINEAL GLAND OF THE SAILFIN MOLLY (*POECILIA VELIFERA*) IN PERIFUSION CULTURE

Darren K. Okimoto
Psychology Department, University of Delaware,
Newark, Delaware, 19716, USA.
phone: 01-302-831-8088 / fax: 01-302-831-3645 /
e-mail: oki@udel.edu

Milton H. Stetson
Biology Department, University of Delaware,
Newark, Delaware, 19716, USA.
phone: 01-302-831-3067 / fax: 01-302-831-2281 /
e-mail: milton.stetson@mvs.udel.edu

Introduction

Rhythmic melatonin production in most teleosts is controlled by circadian oscillators (= biological clocks) located in the pineal organ that are reset everyday by the ambient light:dark (LD) cycle and are capable of rhythmic expression in the absence of light (Bolliet *et al*., 1996; Falcon *et al*., 1989; Iigo *et al*., 1991; Zachmann *et al*., 1992). In this report, we examined rhythmic melatonin release from the pineal gland of the sailfin molly (*Poecilia velifera*) in perifusion culture under different photic conditions.

Methods and Materials

Pineal glands were collected from adults of both sexes and placed in perifusion culture (Fig. 1).

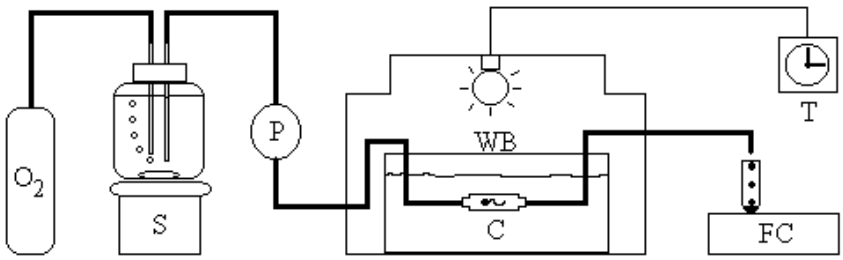


Figure 1. Perifusion culture system. Pineals were placed in up to six tissue chambers (C), with each chamber containing an individual pineal, and submerged in a temperature-controlled water bath (WB). The water bath was housed within a light-tight box containing two 5-watt fluorescent lights. Lighting in the box was set with a programmable electronic timer (T). A peristaltic pump (P) was set to deliver culture medium to each gland at a flow rate of 1.0 ml/hr. Culture medium was kept in a sterile glass reservoir placed upon a magnetic stirrer (S), and continuously stirred and gassed with 95% O_2/5% CO_2 throughout the experiment. Samples were collected with a fraction collector (FC).

Groups of isolated pineals were incubated at 27^{o}C and exposed to: 1) a 12L:12D cycle for 5 days, 2) a 12L:12D cycle for 2 days followed by constant light (LL) for 2 days, and 3) continuous darkness (DD) for 10 days. In addition, 4) another set of glands was sequentially incubated at temperatures initially lower (22^{o}C) and then higher (32^{o}C) than the optimum temperature (27^{o}C) for 4-6 days at each constant incubation temperature to test if the melatonin rhythm (and hence pineal circadian oscillator if present) was temperature-compensated. Melatonin levels in samples were quantified by radioimmunoassay. A periodogram technique using Fourier transform was used to estimate the period (Tau) of the melatonin rhythm in DD.

Results and Discussion

Melatonin release from cultured molly pineals occurs in direct response to the ambient LD cycle. Under 12L:12D, rhythmic melatonin release was observed, with low amounts of melatonin released during the light and high amounts

during the dark. Melatonin release from molly pineals exposed to LL was completely abolished, which may be the result of a light-induced suppression of N-acetyltransferase activity, the rate-limiting enzyme in the melatonin biosynthetic pathway (Falcon *et al.*, 1989). However, under DD, melatonin release was robust and rhythmic with a circadian periodicity ranging between 23.5-24.8 hr, suggesting the presence of a circadian oscillator in the molly pineal. Furthermore, the free-running period of the oscillator driving melatonin production was found to be temperature-compensated over the 10°C range tested (Q_{10} = 0.91).

In conclusion, these data suggest that the molly pineal is directly photosensitive and appears to contain a self-sustaining circadian oscillator regulating melatonin production that is temperature-compensated.

References

Bolliet, V., M. A. Ali, F.-J. Lapointe and J. Falcon. 1996. Rhythmic melatonin secretion in different teleost species: an *in vitro* study. J. Comp. Physiol. B 165: 677-683.

Falcon, J., J. Brun-Marmillon, B. Claustrat and J. P. Collin. 1989. Regulation of melatonin secretion in a photoreceptive pineal organ: an *in vitro* study in the pike. J. Neurosci. 9: 1943-1950.

Iigo, M., H. Kezuka, K. Aida, and I. Hanyu. 1991. Circadian rhythms of melatonin secretion from superfused goldfish (*Carassius auratus*) pineal glands *in vitro*. Gen. Comp. Endocrinol. 83: 152-158.

Zachmann, A., J. Falcon, S. C. M. Knijff, V. Bolliet, and M. A. Ali. 1992. Effects of photoperiod and temperature on rhythmic melatonin secretion from the pineal organ of the white sucker (*Catastomus commersoni*) *in vitro*. Gen. Comp. Endocrinol. 86: 26-33.

ISOFORM EXPRESSION OF NA^+,K^+-ATPASE IN FISH GILLS

Il-Chi Chang,
Institute of Fisheries Science, National Taiwan University
Taipei, Taiwan, R. O. C.

Tsung-Han Lee
Department of zoology, National Chubng-Hsing University
Taichung, Taiwan, R. O. C.

Mei-Jane Fang and Pung-Pung Hwang
Institute of Zoology, Academia Sinica
Nankang, Taipei, Taiwan, 11529, R. O. C.
Phone : 8862-789-9521 / fax : 8862-785-8059 / e-mail :
zophwang@ccvax.sinica.edu.tw

Introduction

The Na^+,K^+-ATPase is an integral protein that responsible for the active transport of ions. This occurs across animal cell membranes and generating an electrochemical gradient which is the driving force of transport system. The Na^+,K^+-ATPase α-subunit contains the catalytic and transport function, and at least three isoform (α1, α2, and α3) are known to exist in mammals. These isoforms are expressed in a tissue-specific manner, and their differential expressions are suggested to be associated with different physiological functions(Jewell. *et al.* 1991).

Through effective mechanisms of osmoregulation, teleosts are able to retain an osmotic and ionic constancy in the internal milieus in hypertonic seawater (SW) or hypotonic freshwater (FW). Gills are the main site responsible for multiple functions, ion secretion in SW-adapted fish and ion uptake in FW-adapted fish. A hypothesis that fish gills may express multiple isoforms of Na^+-K^+-ATPase to perform various functions was proposed.

In this study we studied the expression of Na^+,K^+-ATPase α subunits in the gills of tilapia (*Oreochromis mossambicus* and *Oreochromis nilotica*), carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykisi*s).

Materials and Methods

Gill epithelial cells of SW and FW tilapias, carp and rainbow trout were isolated using percoll discontinuous gradient, and the membrane fractions were obtained by centrifugation. Immunoblotting was conducted by using α1- α2- and α3-specific antibodies, and the results were quantitated by image analysis system.

Results and Discussion

The 3 isoform-specific antibodies of Na^+-K^+-ATPase α-subunit were found to react differentially with the different tissues, e.g., brain, kidneys, heart and gills of all the species tested, and the results revealed a tissue-specific distribution of the 3 isoforms. The α1-specific antibody detected a major band with relative molecular masses of about 100 kDa in brains, kidneys and gills of tilapia, rainbow trout and carp, and only a minor reaction in the hearts. In the case of α3-specific antibody, strong reaction was found in the hearts of all species, but only a minor band in the other tissues. On the other hand, the α2-specific antibody failed to detect any major bands in the tissues tested.

Gill tissues showed positive reactions to the antibodies against α1 and α3 isoforms in all the species studied (Fig.1, 2), suggesting at least two isoforms, α 1-like and α3-like, of Na^+,K^+-ATPase are expressed in fish gills. Moreover, expression of α1-like isoform was salinity-dependent while that of α3-like was not. In the case of tilapia, *O. mossambicus*, the amount of Na^+,K^+-ATPase α1-like in SW group was significantly higher (about 3.5 folds) than that in FW group, but no significant difference in the amount of α3-like subunit was found between the two groups ($p>0.05$, t-test). Consequently, the ratio of α1-like/α3-like in SW group was also higher than that in FW.

Fig.1 Immunoblot of α1-like isoform of Na^+,K^+-ATPase in fish gills. P, positive control (rat brain); M, *O. mossambicus* ; N, *O.nilotica*; C, carp; R, rainbow trout; FW, freshwater; SW, seawater.

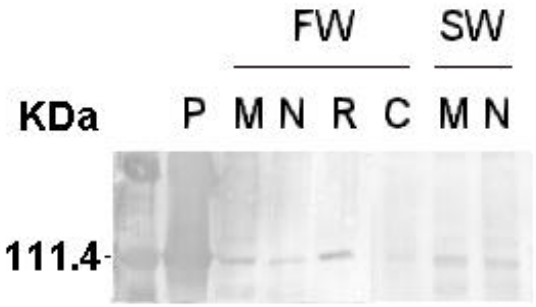


Fig.2 Immunoblot of α1-like isoform of Na^+,K^+-ATPase in fish gills. P, positive control (rat brain); M, *O. mossambicus* ; N, *O.nilotica*; C, carp; R, rainbow trout; FW, freshwater; SW, seawater.

In early studies, Motais (1970) postulated that there were two different physiological forms of gill Na^+,K^+-ATPase (actinomycin-D sensitive and actinomycin-D insensitive) in freshwater-adapted eels. Beckman et al.(1990) found similar results in chinook salmon (*Oncorhynchus tshawytscha*). In 1991, Pagliarani et al., found that the affinities for ATP, Na^+, K^+ and ouabain of Na^+-K^+-ATPase were different in freshwater- and brackish water-acclimated

rainbow trout (*Oncorhynchus mykiss*) and suggested this as habitat-dependent characters. All these studies implied that there might be two isoforms of Na^+,K^+-ATPase in fish, but no direct evidence is available so far. The present study for the first time reported that at least two isoforms of gill Na^+,K^+-ATPase, which are involved in the ion- and osmo-regulation mechanisms, exist in gills, and suggested that differential expressions of two isoforms may be associated with different functions, secretion and uptake of ions in the gills of SW- and FW-adapted fish.

Acknowledgments

The authors are grateful to Dr. Douglas M. Fambrough, Dr. Thomas A. Pressley and Dr. Kathleen J. Sweadner for providing the antibodies. This study was supported by grants to P.P.H. from the National Science Council, Taiwan, R.O.C. (NSC 85-2311-B001-067 and NSC 86-2311-B001-066.

References

Beckman, B. R., and Zaugg, W. S. 1990. Effect of actinomycin-D on gill Na+,K+-ATPase of juvenile chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). *J. Fish Biol*. 37:907-911.

Jewell, E.A., and. Lingrel, J. B.1991. Comparison of the substrate dependence properties of the rat Na,K-ATPase α1, α2, and α3 isoforms expressed in Hela cells. J. Biol. Chem. 266:16925- 16930.

Motais, R. 1970. Effect of actinomycin-D on the branchial Na-K dependent ATPase activity in relation to sodium balance of the eel. *Comp. Biochem. Physiol*.34: 229-236.

Pagliarani, A., Ventrella, V., Ballestrazzi, R., Trombetti, F., Pirini, M., and Trigari, G. Salinity- dependence of the properties of gill Na^+,K^+ATPase in rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol.* 100B (2): 229-236.

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION

OF SOME BIOLOGICAL TRAITS OF SALMO IRIDEUS G.

Yordan Staykov
Dept. of Aquaculture, Thracian University
6000 Stara Zagora, Students town, Bulgaria
phone/fax +35942/77036, e-mail: staykov@uzvm.uzvm.bg

Alexander Blagoev
Dept. of Agricultural Engineering, Thracian University

Svetlin Tanchev
Dept. of Animal Genetics, Thracian University

Abstract

The study included female and male trout of *Salmo irideus* G. The aim of the experiment was phenotypic characterization of the fertility of trout, spawned for the first time. The effect of parents on the body length of the progeny of this fish was determined. Female trout have a good relative fertility (2182±111.9 eggs) and male fish - high quality of sperm liquid (concentration 11.42±0.28 mln per cub mm, and activity 55.42±1.46 s, and mobility 69.17±0.87% of spermatozoa). The effect of the female trout on the phenotypic and genotypic variety of live weight and body length of the progeny is considerably higher compared to the male parents.

Keywords: rainbow trout, fertility, sperm, influence phenotype and genotype.

Introduction

Qualitative indices of sexual products received from fishes are important indications for the reproductive ability of the fishes. The possibility to use the size of the eggs as a specific indication of species has already been proved. The size of eggs can be considerably different between separate population of the same species (Galkina, 1970). There are considerable individual variations in the average rate of female specimen eggs size from the same population

(Vaselevski, 1980). These indices depend on the age, size, and physiological condition of the female specimen (Kazakov, 1981).

Zuromska et al. (1984) ascertained the fact that males have bigger influence during the embryonal period, while females have bigger influence during the stage of fry. The influence of the size of eggs on fry weight is bigger than the influence on their length (Todorov et al., 1983). Rainbow trout fries from female fishes at the same age with equivalent weight but from small and from large eggs are not different by size and rate of growing from one another. (Privolnev, 1964).

Kazakov et al., (1980); Graham et al., (1988) proved that there is an influence of size of eggs on the process of growth only during the period of transition to mix feeding.

Size of the progeny depends on age of breeding female and size of eggs (Gall, 1974) but not on the size of the parents (Bondari et al., 1985). Shindavina (1987) studied the influence on idiosyncrasy of breeding males and females on the survival rate of embryo and fries of rainbow trout. She found that characteristics of separate females and size of eggs do not have influence on length of embryo period. The influence of breeding female on fries weight becomes stronger in transition of active nutrition. (Shindavina et al., 1987).

The aim of this study was phenotypic characterization of the reproductive abilities of breeding females and males of *Salmo irideus* G., used for the first time in spawning and their effect on the phenotypic and genotypic diversity of progeny assessed in respect to the characteristics of live weight and length of the body.

Material and Methods

The study was carried out in State Hunting Farm “Bukovets”, Tvarditsa. For phenotypic characterization of the reproductive abilities of breeding females and males, the study included 12 females and 12 males fishes caught by chance. They were spawned for the first time. Their sexual products were received by massage. The lengths of the body (cm) and the live weights were measured at the same time. Capacity of the eggs was measured with graduated measure. Number of eggs was fixed by volumetric method. The relative fertility was calculated too. One hundred roe were taken from each breading female. We

measured their diameter d_1 and d_2 (mm) with microscope stereoscope MBC-1 and their weight (mg) with torsion scales TV-I. The volume of sperm liquid was measured with graduated measure. Concentration of the sperm (mln per cub mm), activity (s) and mobility (%) of spermatozoa were calculated by routine methods (Semkov et al., 1988). PH of sperm liquid was determined by pH-meter MV-81.

Six male and five female breeding fishes were included in the experiment to study the effect of the parents on the genotypic diversity of the characteristics of body length and live weight. One hundred eggs were taken from each 3 years old female fish. They were inseminated with 2 ml sperm liquid, received from one 5 years old breeding male using the dry method of insemination (exp. 1). In the second half of the experimental scheme, one 5 years old breeding female was used. Its eggs were separated into 4 experimental groups. Each of them contained one 100 eggs. Eggs from different groups were inseminated with 2 ml sperm liquid from 4 different 3 years old breeding males (exp. 2). Incubation of eggs from each experimental variant was done in special incubation apparatus (Californian type) with equal hydrochemical conditions. On the 30^{th} day after incubation body lengths (mm) and live weights (mg) of 50 fries from each group were measured.

The coefficients of genotypic diversity (h^2) were calculated and analyzed. All of the data was analyzed by descriptive statistical methods (Snedekor, 1961; Plohinski, 1964; Becker, 1968).

Results and Discussion

Average values of investigated characteristics of breeding rainbow trout females are represented on table 1. The data shows that live weight and body length are comparatively low. Variation of the specimens especially by live weight is impressive. Confirmation of this fact is high value of variation coefficient for this parameter (C=30.17%). The absolute fertility of rainbow trout, used for the first time in the spawning is good (table 1). Comparison between experimental groups and farm broodstock (Todorov, 1988) by relative fertility shows growing with 43.43%.

Variation of fertility is considerable big (C=19.21%). This fact shows possibility to make selection of breeding females by this productive character.

Low values of size and weight of the eggs are typical for fishes of this age (Table 1). Variation of the characteristics is insignificant.

Table 1. Phenotypic Characterization of the Characters Studied in Breeding Females of Rainbow Trout

Characters	n	$\bar{x}\pm S\bar{x}$	C
Live weight, g	12	476.67 ± 42.30	30.71
Length of the body, cm	12	33.08 ±1.22	12.79
Absolute activity, No	12	1000.52 ±55.55	19.21
Relative fertility, No.	12	2182.02 ±111.09	17.62
d_1 of eggs, mm	12	3.89 ±0.01	6.77
d_2 of eggs, mm	12	4.23 ±0.01	6.62
Weight of eggs, mg	12	38.16 ±0.07	6.89

Table 2. Phenotypic Characterization of the Characters Studied in Breeding Males of Rainbow Trout

Characters	n	$\bar{x}\pm S\bar{x}$	C
Live weight, g	12	476.75 ± 25.66	18.17
Length of the body, cm	12	33.71 ±0.98	9.70
Concentration of spermatozoa, mln/cub mm	12	11.42 ±0.28	8.20
Activity of spermatozoa, s	12	52.42 ±1.46	9.30
Mobility of spermatozoa, s	12	69.17 ±0.87	4.20
pH of sperm liquid	12	7.98 ±0.04	0.60

Table 3. Live Weight and Length of Fry Body

Experimental group	n	Live weight, mg		Fry body length, mm	
		$\bar{x}\pm S\bar{x}$	C	$\bar{x}\pm S\bar{x}$	C
	I experiment				
1	50	125.10 ± 1.08	6.11	24.96 ± 0.17	4.76
2	50	149.63 ± 1.99	9.41	24.96 ± 0.14	4.02
3	50	130.19 ± 1.59	8.63	24.66 ± 0.13	3.80
4	50	190.60 ± 1.63	6.04	25.72 ± 0.12	3.43
5	50	153.05 ± 1.23	5.68	25.27 ± 0.07	2.06
	II experiment				
1	50	220.58 ±1.62	5.20	26.37 ± 0.06	1.70
2	50	219.32 ±2.60	8.40	26.45 ± 0.06	1.64
3	50	221.36 ±1.63	5.21	26.50 ± 0.06	1.58
4	50	216.84 ±1.59	5.18	26.30 ± 0.06	1.62

Live weight and body size of the breeding males used is lower than the requirements for this category of fish (Table 2). Equality of researched traits is good. Variation of traits is lower for female specimens. Assessment of sperm liquid shows very good qualitative indices.

Values for concentration of sperm liquid, activity and mobility of spermatozoa confirm this (Table 2). Results describing the effect of breeding fishes on live weight and body length of fries are shown in Table 3.

The progeny in experiment 1 is received from five females and one male, and from one female and four males in exp. 2.

The comparison of the results shows that phenotypic diversity of studied traits of the specimens from experiment 1 is bigger. Coefficients of inheritability, characterizing genetic determinance of variation on studied traits are shown in table 4. Coefficients of inheritability (h^2) are higher in the first experimental case. Some of them (calculated by the Becker method, 1968) are even bigger than 1 (h^2=3.397). This fact allows the conclusion that a high genetic diversity is determined from big differences between different groups of broodstock.

There was not a genetic diversity in experiment 2 (Table 4). The results for inheritability of specific characteristics in live weight and body length of fries corresponded with phenotypic diversity of fries from the 2 experimental groups.

Table 4. Coefficient of Genetic Diversity (H^2) in Respect to the Traits of Fry Live Weight and Length of Body of Rainbow Trout

Characters and sources of variation	dr	ms	After Becker $h^2 \pm S^{h^2}$	After Plohinski $h^2 \pm S^{h^2}$	F
	I experiment				
Larval live weight:					
- between groups	4	371.004	$3.397 \pm 2.882^{+}$	$0.822 \pm 0.009^{+}$	282.41
- within groups	245	118.176			
Larval body length:					
- between groups	4	8.004	$0.565 \pm 0.712^{+++}$	$0.431 \pm 0.014^{+++}$	9.220
- within groups	245	0.868			
	II experiment				
Larval live weight:					
- between groups	3	195.591	$0.006 \pm 0.084^{+}$	$0.016 \pm 0.015^{+}$	1.070
- within groups	196	182.379			
Larval body length:					
- between groups	3	0.283	$0.041 \pm 0.191^{+}$	$0.023 \pm 0.015^{+}$	1.520
- within groups	196	0.186			

This fact allows the conclusion that breeding females have stronger effect on genotypic and phenotypic diversity of studied characteristics. Zuronska, Markowska (1984) and Gall (1974) have the similar opinion.

Conclusions

Values characterizing live weight and body length of breeding males and females of the population studied *Salmo irideus* G., used for the first time in spawning are low. There is considerable phenotypic diversity of the live weight for males and females.

Breeding females are characterized with a high relative fertility, while breeding males are characterized with high qualitative indices of the sperm liquid.

Breeding females have a higher effect on phenotypic and genotypic diversity of the characteristics of live weight and body length of progeny than breeding males.

References

Becker, W. 1969. Manual of Procedures in Quantitative Genetics. *Wash. State Univ*. W.

Bondari, K., G. Ware, B. Mullinek and J. Joyce. 1985. Influence of brood fish on the breeding performance of channel catfish. *Progr. Fish Culturist.* 47. 1:21-26.

Fraham, A. 1988. Status of quantitative genetics and stock improvement for rainbow trout. *Nat. Breeders Round Table*. 117.

Galkina, Z. 1970. Influence of the age of breeding females of *Salmo salar* L. and *Salmo irideus* G. on size of the eggs. *Quest. of Ichthyology*. 10, 5(14):827-837.

Gall, G. 1974. Influence of eggs and size of female on hatchability and growth in rainbow trout. *Calif. Fish and Game*. 26-35.

Kazakov, P and M. Melnikova. 1981. Size and weight of the fries of Atlantic salmon (*Salmo salar* L.) according to size of the eggs. *Sc. papers. GosNIORH*. 153:58-70

Kazakov, R. 1981. The effect of size of Atlantic salmon (*Salmo salar* L.) eggs on embryos and juveniles. *J. Fish. Biol*. 19. 453.

Plohinski,. N. 1964. Heredity. Novosibirsk. *Ac. of Sc. USSR*.

Privolnev,. T., Z. Galkina and G. Galkin. 1964. Influence of the size of breeding males and size of the eggs on progeny of rainbow trout. *News GosNIORH*. 58:159-166.

Semkov, M., I. Nikolov and S. Georgiev. 1989. Manual of artificial insemination in animal husbandry. Sofia. Zemizdat.

Shindavina, N. 1987. Incubation time of fries of rainbow trout according to idiosyncrasy of breeding fishes. *Sc. pap. GosNIORH*. 263:11-16.

Shindavina, N. and V. Nikandrov. 1987. Estimation of breeding fishes of rainbow trout *Sc. pap. GosNIORH*. 259:11-16.

Snedekor, G. 1961. Statistical methods in agriculture and biology. Moscow. Selhozizdat.

Todorov, M, E. Ivancheva and A. Vladimirov. 1983. Research of the size and weight of eggs and fries of rainbow trout. *Sci. pap. of Agr. Fac*. vol. XXX.

Todorov, M., Y. Staykov and S. Georgiev. 1988. Study of the breeding females of *Salmo irideus* G. *Firs Sc. Conf. of Young Sc. Workers*. St. Zagora.

Vaselevski, G. 1980. Weight research on the eggs of rainbow trout. *Fish industry*. 7.

Zuromska, H. and J. Markowska. 1984. The effect of sexual products quality on offspring survival and quality in tench (*Tinca tinca*). *Pol. Arch. Hydrobiol*. 31. 3:287-313.

THE BLOOD HEMOGLOBIN CONCENTRATION

OF TRIPLOID BROOK TROUT

Evelyn J. Stillwell
University of New Brunswick, Department of Biology,
Bag Service # 45111
Fredericton, N.B., Canada E3B 6E1
Phone: 506-447-3313; Fax: 506-453-3583;
E-mail: estillwe@unb.ca

Tillmann J. Benfey
University of New Brunswick, Department of Biology

Introduction

Triploid fish have certain advantages which make them appealing for both fisheries management and aquaculture. The sterility of triploid fish eliminates concerns about the interbreeding of domestic and wild stocks of fish, while the lack of sexual maturation in triploid females minimises the declines in flesh quality, immunocompetence, and growth rate that are normally seen in diploid fish during sexual maturation (Benfey, 1998). Despite having these advantages, triploid fish are not cultured extensively because their performance is reported to be inferior to that of diploids under conditions of high biological oxygen demand and/or low oxygen availability (Benfey, 1998).

It has been suggested that the impaired performance of triploids when oxygen is limiting might be attributable to an altered blood-oxygen transport capacity. Blood hemoglobin concentration (BHC) is a basic measure of the ability of blood to transport oxygen and, as such, an indicator of an organism's ability to supply oxygen adequately for metabolic processes. Interestingly, studies which have examined BHC have alternately reported triploid BHC to be equivalent to, higher than, and lower than that of diploids (Benfey, 1998). The objective of the present study was to examine the BHC of diploid and triploid brook trout (*Salvelinus fontinalis*) in an attempt to understand the inconsistencies reported in the literature.

Methods

BHC was measured every 3-4 months over a two-year period in an all-female (n=32) and a mixed-sex (n=50) group of diploid and triploid brook trout. Within each group, diploids and triploids came from the same egg lots and were exposed to identical rearing regimes; triploidy was induced by hydrostatic pressure treatment of eggs shortly after fertilization. On each sampling date fish were anaesthetized and a small blood sample (£ 100 1l) was collected via caudal vessel puncture. BHC was determined spectrophotometrically in duplicate for each fish using a commercially-prepared kit (Sigma Chemical Co., 525-A).

Results and Discussion

The BHC was found to fluctuate significantly over time suggesting that age and/or season affected this parameter. Although the BHC was generally similar in all sex and ploidy groups, certain differences were observed: around the time of ovulation diploid females had a significantly lower BHC than their male and triploid counterparts (Figures 1 and 2). Our results suggest that the BHC differences noted were not related to ploidy or sex differences per se but appear instead to reflect endocrinological/physiological differences between these groups.

The reproductive endocrinology of triploid females is dramatically different from that of diploid females: the ovaries of triploid females produce no significant amounts of 17ß-estradiol and these fish remain sexually immature. Ploidy-related differences in female reproductive endocrinology are most pronounced in the pre-spawning period when levels of sex steroids become significantly elevated in diploid females but remain negligible in triploid females. Relative to females, the reproductive endocrinology of males is much less affected by the induction of triploidy: triploid males produce normal levels of sex steroids and show normal patterns of sexual maturation.

Estrogens are potent inhibitors of erythropoiesis (Blobel et al., 1995). As erythrocytes develop and mature they synthesize hemoglobin (Fänge, 1986). Estrogen-related erythropoiesis inhibition could thus produce a reduction in BHC. However, since fish erythrocytes can survive for up to 150 days in circulation (Hevesy et al., 1964), this effect may not be immediate. The influence of estrogens on erythropoiesis and hemoglobin synthesis may explain

why the BHC decreases in diploid females at, or following, spawning while no such decrease is evident in triploid females or in males of either ploidy.

Estrogens also stimulate the production of vitellogenin in female fish (Sumpter, 1984). This yolk-protein has been shown to bind iron (Tsioros et al., 1996). Consequently, during vitellogenesis, the amount of iron available for hemoglobin sythesis in the female may be reduced. Energetically, vitellogenesis is also an extremely demanding process. The iron-binding capacity of vitellogenin combined with the metabolic demands of vitellogenesis may thus create physiological conditions which favour the suppression of hemoglobin synthesis in diploid females. One would not expect this situation to arise in diploid males or in triploids of either sex since these fish produce no significant amount of vitellogenin (Benfey, 1998).

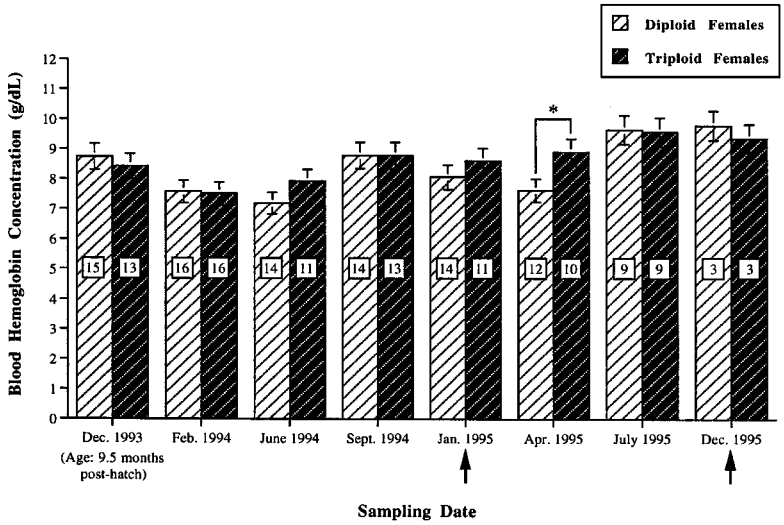


Figure 1. Blood hemoglobin concentrations in an all-female group of diploid and triploid brook trout; bars represent means (± 1 standard error). Sample sizes are indicated by the numbers on the histogram. * represents a marginally significant difference (p£0.0131). Sampling dates marked with arrows indicate dates when diploids were "spawned" (i.e., stripped of eggs).

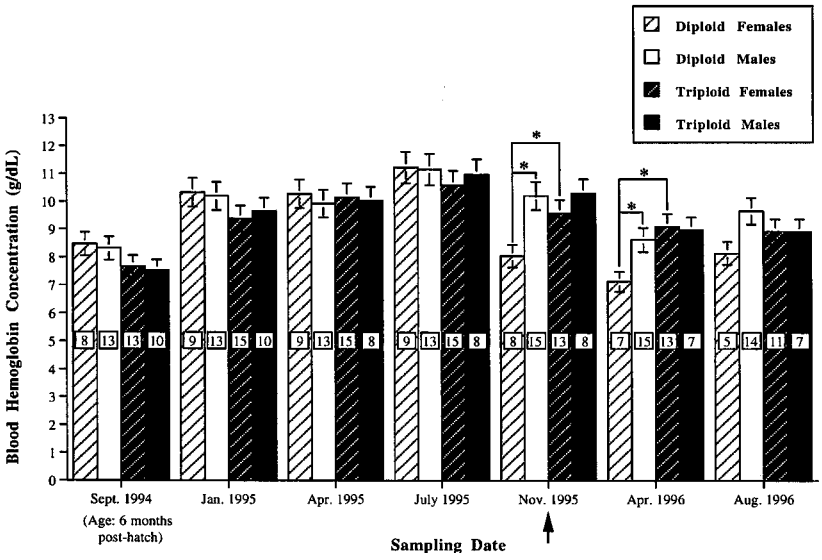


Figure 2. Blood hemoglobin concentrations in a mixed-sex group of diploid and triploid brook trout; bars represent means (± 1 standard error). Sample sizes are indicated by the numbers on the histogram. * represents a significant difference (p£0.0018). The sampling date marked with an arrow indicates a date when diploids were "spawned" (i.e., stripped of gametes).

The overall similarity in the BHC profiles of diploids and triploids suggests that ploidy has little effect on BHC. The differences noted appear instead to reflect intermittent endocrinological/ physiological differences between groups. Although not the definitive measure of oxygen carrying capacity, our BHC results suggest that the blood of triploid fish should be as effective as that of diploids in meeting biological oxygen requirements, and that triploid performance should not be restricted by blood-oxygen transport capacity.

Acknowledgements

This research was funded by a grant from the Natural Sciences and Engineering Research Council of Canada. The experimental protocol was approved by the UNB Animal Care Committee, meeting Canadian Council of Animal Care Guidelines.

References

Benfey, T.J. 1998. The physiology and behavior of triploid fishes. Rev. Fish. Sci.: In Press.

Blobel, G.A., C. Sieff and S.H. Orkin. 1995. Ligand-dependent repression of the erythroid transcription factor GATA-1 by the estrogen receptor. Molec. Cell. Biol. 15: 3147-3153.

Fänge, R. 1986. Physiology of Haemopoiesis. In: Fish Physiology: Recent Advances. pp.1-23. Edited by S. Nilsson and S. Holmgren. Croom Helm Ltd., London.

Hevesy, G., D. Lockner and K. Sletten. 1964. Iron metabolism and erythrocyte formation in fish. Acta Physiol. Scand. 60: 256-266.

Sumpter, J.P. 1984. The seasonal reproductive cycle of the rainbow trout. In: Endocrinology: Proceedings of the 7th International Congress of Endocrinology. pp. 793-796. Edited by F. Labrie and L. Proulx. Excerpta Medica, Amsterdam.

Tsioros, K.K., J.A. Holmes and J.H. Youson. 1996. Origin of iron in sea lamprey, *Petromyzon marinus*, tissues during embryogenesis and early larval life. Can. J. Zool. 74: 2206-2210.

SEX DETERMINATION OF ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*), WINTER FLOUNDER (*PSEUDOPLEURONECTES AMERICANUS*) AND HADDOCK (*MELANOGRAMMUS AEGLEFINUS*) USING ULTRASONOGRAPHY

D.J. Martin-Robichaud
[1]Department of Fisheries and Oceans
Biological Station, St. Andrews, N.B., Canada E0G 2X0
(506)529-8854; fax (506)529-5862
martin-robichaudd@mar.dfo-mpo.gc.ca

Melissa Rommens
[1]Department of Fisheries and Oceans
rommensm@mar.dfo-mpo.gc.ca

Luke Vallee
University of New Brunswick
Department of Biology, Fredericton, N.B., Canada

Introduction

The ability to assess the sex and stage of maturity of finfish is essential for fisheries management and aquaculture applications. Various biochemical and biopsy techniques are used to sex live specimens, however, the procedures are costly, lengthy and not easily adaptable for industry use. A fast, non-invasive technique to sex fish and to determine gonadal maturity would be useful for sexually monomorphic fish.

The purpose of this study was to evaluate the feasibility of using ultrasonography to determine the sex of juvenile halibut, and mature halibut, haddock and flounders throughout the year. Emphasis was placed on describing the methodology and image characteristics to aid the conduct, evaluation and interpretation of ultrasound images by others.

Materials and Methods

An ATL Ultramark 4 Plus ultrasound with an Access mechanical multi-frequency sector scanhead (5.0, 7.5 and 10 MHz) was used. Acoustic energy is absorbed or reflected back to the transducer as it passes through tissues. Higher frequency acoustic signals result in greater resolution but less depth penetration. The amount of signal reflected back to the transducer is dependent on the density of the tissues. Dense tissues reflect more sound waves and appear white whereas less-dense, more fluid-filled organs and tissues similar to water reflect less and the image is darker.

Initial ultrasound scans were conducted on ten wild-caught, mature winter flounder and six wild-caught juvenile halibut (52 to 67 cm FL). The fish were dissected to confirm the assessments determined by ultrasound. The ultrasound scanhead was suspended in the water about 1-3 cm above the fish. The transducer frequency was 5 MHz and penetration depth set at 75-90 mm. Cross-sections of the gonads were scanned directly posterior to the gut region. These initial images were used as a reference to determine the sex of four year old cultured juvenile halibut (n=31) (54.8 to 71.2 cm; 2.3 to 5.2 kg) with ultrasound.

Mature Atlantic halibut (females; 96 to 120 cm, 13.3 to 28.4 kg; males; 82 to 105 cm, 6.1 to 19 kg) of known sex were scanned with ultrasound in July 1997 after spawning and May 1998 during the spawning period. Halibut were placed on a neoprene-covered table 6-12 cm underwater. The scanhead was suspended in water approximately 1-7 cm above the surface of the fish. The transducer frequency was 5 MHz and penetration depth set at 110-120 mm for an overall image of gonad morphology. To increase the resolution and to obtain magnified images of individual oocytes in spawning fish the transducer frequency was set at 7.5 MHz and 40 mm depth.

Mature haddock (average 62.4 cm and 2.1 kg) were scanned with ultrasound every 4-6 weeks from January 20, 1997 to April 23,1998. While anaesthesized the haddock were positioned and held stationary with the ventral surface up. The scanhead was suspended 1-4 cm in the water over the ventral surface of the fish and positioned directly anterior to the urogenital pore.

Results

Since flatfish are positioned lying on one side the image shows one gonad above the other separated by the median mesentery and vertebral spinal rays. The ovary of immature halibut appear as two whitish-gray, granular lobes, one lying over the other (fig. 1A). The testes of juvenile halibut are more difficult to identify due to their small size therefore sex determination of immature halibut is based primarily on the presense or absence of ovarian lobes.

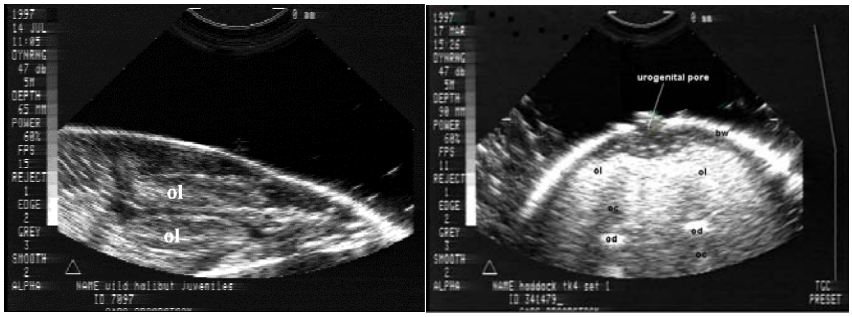


Figure1A. Female halibut juvenile. 53.6 cm FL. Ovarian lobes (ol); B. Mature haddock female during spawning. The ovarian lobes (ol) shown as white oval structures lie above the ventral body wall (bw). The darker area within each lobe is the ovarian cavity filled with ovarian fluid. The white dots are oviducts (od).

In prespawning mature female halibut the large ovarian lobes appear positioned one over the other with a characteristic oval shape and granular appearance. As with the juveniles, testes are more difficult to detect but may be identified as dark, fluid-filled bilobed structures. Often the vas deferens can be detected as a small, white structure in the middle of each lobe. In spawning females a higher frequency results in an image which clearly shows highly reflective, white individual oocytes surrounded by unreflecting, dark areas of ovarian fluid.

The ovaries of mature haddock females scanned over the posterior ventral surface were seen as two round dense granular lobes adjacent to one another positioned over the ventral surface of the highly reflective swimbladder wall

(fig. 1B). During spawning the ovarian cavity containing ovarian fluid was sometimes detected as a slightly darker area within the ovary. The testis of haddock lie close to the swimbladder wall and were identified by their dark, fluid-filled lobes. The vas deferens was frequently visible, especially during the spawning season.

Discussion

Ultrasound has the potential to be a valuable tool for broodstock management and for monitoring gonadal development during reproductive studies. However, successful interpretation of ultrasound images and correct use of ultrasound equipment depends on experience. Martin et al. (1983) provides a good description of the principles of ultrasonography and it's application to fish. Understanding the internal morphology of fish, especially flatfish, in relation to the image orientation is critical for accurate interpretations. We have emphasized the ideal scanning location to acquire good gonadal images for ease of interpretation. Haddock gonads, like those of Atlantic cod, develop caudally and anteriorly (Karlsen and Holm, 1994). Therefore, the maximum diameter of the ovary is at the posterior end of the body cavity so it is easier to observe the ovary by scanning the area directly anterior to the urogenital pore.

Testis of all species examined were the most difficult to identify. Compared to the white, granular ovarian tissue of a mature ovary, testes appear darker due to the higher water content. Reimers et al. (1987) also found that closer to spawning and during spermiation the testes are lighter and more granular due to the presence of spermatozoa.

The gonads of juvenile fish are small and can easily be missed if the transducer is passed over the critical area too quickly. Using a medium frequency transducer (5 MHz) it is possible to sex juvenile halibut accurately. The ovaries of juvenile halibut are readily observed directly posterior to the gut (fig.1A). Using ultrasound the size of gonads observed in relation to fish size can be used as a criteria for determining the sex of fish. For example, immature female salmon are identified by the presence of ovaries but immature males are determined by exclusion (Mattson, 1991; Reimers et al., 1987). Similarily the sex of male juvenile halibut is frequently assumed from the absence of distinct ovarian lobes.

For good quality halibut eggs it is critical to obtain eggs from halibut broodstock within 6 h of ovulation to prevent overripening of oocytes. With experience it

may be possible to evaluate the stage of oocyte development using ultrasonography. At 7.5 MHz individual oocytes can easily be seen in the ovarian cavity and the distribution and density of ovulated eggs in the ovarian cavity may indicate the timing of ovulation. The presence of significant quantities of ovarian fluid surrounding large, hydrated oocytes indicates that ovulation has commenced (Shields et al.,1993).

These results indicate that ultrasonography is a suitable technique for sex determination of juvenile halibut and mature flatfish and haddock. The applications for ultrasound technology in broodstock management are diverse. Selecting juvenile fish based on sex for future broodstock limits the biomass required to be cultured to maturity. Many culture operations still rely on wild-caught broodstock and rapid assessment of sex of newly captured fish would be useful. The ability to sex gadids such as cod and haddock which are volitional spawners permits the stocking of specific sex ratios into spawning tanks. Using ultrasound to determine the timing of ovulation in batch spawning fish such as halibut would be a valuable technique for ensuring good, quality eggs. However, further studies monitoring female ovulation cycles with ultrasound is required. Using ultrasound on mature halibut underwater to quickly evaluate ovarian development would be much less stressful and invasive than the present practice of applying pressure to the ovary to extrude eggs for evaluation.

References

Karlsen, O. and J.C. Holm. 1994. Ultrasonography, a non-invasive method for sex determination in cod (*Gadus morhua*). J. Fish Biol. 44:965-971.

Martin, R.W., J. Myers, S.A. Sower, D.J. Phillips and C. McAuley. 1983. Ultrasonic imaging, a potential tool for sex determination of live fish. North Am. J. Fish. Manage. 3:258-264.

Mattson, N.S. 1991. A new method to determine sex and gonad size in live fishes by using ultrasonography. J. Fish Biol. 39:673-677.

Reimers, E., P. Landmark, T. Sorsdal, E. Bohmer, and T. Solum. 1987. Determination of salmonids' sex, maturation and size: An ultrasound and photocell approach. Aquaculture Magazine Nov/Dec, 41-44.

Shields, R.J., J. Davenport, C. Young and P.L. Smith. 1993. Oocyte maturation and ovulation in the Atlantic halibut, *Hippoglossus hippoglossus* (L.), examined using ultrasonography. Aquacult. Fish. Manage. 24:181-186.

EXTENDER-CRYOPROTECTANTS

FOR STRIPED BASS SPERMATOZOA

L. Curry Woods III and Karen M. Jenkins
Crane Aquaculture Facility
Department of Animal and Avian Sciences
University of Maryland
College Park, Maryland 20742-2311 USA

Introduction

Cryopreservation of sperm is a widely used practice in animal husbandry that is now being intensively studied and utilized for many aquaculture species. The increasing popularity of the striped bass as a favorite species for the global aquaculture industry, coupled with the current inability to cryopreserve large amounts of milt efficiently, provide the impetus for this study. Specifically, difficulties have been shown in obtaining motile spermatozoa post-thaw in all previously published papers on cryopreservation of striped bass sperm. Our study was initiated to develop a successful extender and cryoprotectant which would allow activation and motility of the sperm post-thaw.

Methods

Physical and Chemical Characterization:

Milt was collected from striped bass produced at the Crane Aquaculture Facility (CAF). Fish from three different domestic generations and five yearclasses over the course of two years were sampled. All fish were anesthetized to surgical plane prior to collection. Care was taken during milt collection to avoid water, urine and fecal material. Color, viscosity, and the total expressed volume of milt were noted at collection. Four mL of blood was drawn from the caudal vein following milt collection. Immediately after collection, sperm was tested for activation with deionized, ultra-filtered water (DIUF, Fisher Scientific) with an osmolality of $\leq$ 5 mOsm/kg. The percentage active sperm and the active motility time for each sample was recorded using a Hitachi video camera (Model: KP-140) for later analysis and repetitive, objective quantification. The

percentage live sperm was determined with differential staining using eosin and nigrosin and a Zeiss Phase Microscope (Model: 470916/9902BI @ 400x). The number of sperm cells per mL was determined with a Makler counting chamber. Seminal plasma was separated from neat milt samples using a Heraeus refrigerated centrifuge (Model: Labofuge 400R @14,000 rpm for 30m). Blood plasma was collected with an Eppendorf centrifuge (Model: 5415C @10,000 rpm for 5m). Osmolality and pH were measured in neat milt, seminal plasma, blood plasma, and urine with a Wescor vapor-pressure osmometer (Model: 5520) and an Orion micro electrode (Model 9810BN) respectively. The concentrations of Na^+ and K^+ in the seminal plasma, were measured using a Perkin-Elmer atomic-absorbtion spectrophotometer (Model: 5100PC).

Extenders:

Three extenders were utilized during the two spawning seasons and are presented below in Table 1. Milt was extended 2:1 using freshly collected samples and thereafter, continuously stored at 2-4^{o}C. The extended milt samples were examined for percentage active sperm, the active motility time, and percentage live sperm after: being initially extended, 24h, 48h, and 1week.

Cryopreservation:

Two extenders were utilized in a preliminary cryopreservation experiment. Both extenders were added to freshly pooled milt as described above. The cryoprotectant dimethyl sulfoxide (Sigma DMSO-4540) was added at 5.0% and 7.5% of the total volume, mixed, and the mixture was loaded into 0.5mL plastic straws. Two freezing rates, -30^{o}C/m to -120^{o}C and -40^{o}C/m to -120^{o}C, were developed and implemented using a Planer controlled-rate cryogenic freezer (Model: Kryosave-PC/KS30). The samples were held at -120^{o}C for two minutes in the freezer, loaded into coded goblets and/or canes and plunged into liquid nitrogen. The samples were maintained at -196^{o}C in a Taylor-Wharton dewar (Model: 35VHC) as part of the CAF gene bank for post-thaw evaluation.

Table 1. Extenders for short-term storage of striped bass milt and for the base ingredient of cryogenic media for freezing.

Extender	Composition Ingredient	Amount
(A) "8:1 Na:K"	H_2O w/5% Dextrose	200mL
pH=7.6	NaCl	0.500g
-	$KHCO_3$	0.065g
(B) - "Stein's"	H_2O	200mL
pH=7.6	NaCl	1.500g
-	NaHCO3	0.400g
-	KCl	0.080g
-	egg yolk	40mL
(C) - "Brown's $^{\#}$13"	H_2O	200mL
pH=7.6	NaCl	1.720g

Frozen straws were thawed by plunging into a 25^{o}C water bath. The post-thaw sperm were immediately evaluated for activation and motility. Adequate samples were frozen from each extender x cryoprotectant x freezing rate treatment to allow evaluations to take place after: 24h, 1week, and 1 year.

Results

The range of live sperm cells (differential staining) immediately after collection ranged from 80-100% across a typical six-week spawning season. Within the high viability samples (i.e. 95-100% live), the percentage active sperm ranged between 90-100%. The range of active motility time with fresh striped bass sperm was 30-45s. The amount of expressed milt varied according to size and/or age of the fish but was not statistically different between filial generations based on a relative volume (mL milt/kg body weight).

Osmolality and pH of the milt ranged between 329-361 mOsm/kg and between 7.74-8.18 respectively. Sperm density varied from 8-12 x 10^{10} cells/mL of milt.

Pooled seminal plasma samples had concentrations of Na^+ and K^+ of 3.3g/L and 0.8g/L respectively. To date, motile sperm have been found at all intervals checked for both cryogenic media and both freezing rates, however, both the percentage active sperm and the active motility time were significantly less than the neat milt samples. These results are encouraging in that they report for the first time, significant numbers of motile striped bass sperm post-thaw. Further study is required however, to improve the number of cells which survive and remain viable post-freeze/thaw and to demonstrate that these motile cells in fact are capable of fertilizing viable eggs.

OF EGGS, LARVAE, AND YOUNG OF WOLFFISH,

A PERSPECTIVE SPECIES FOR COLD-WATER AQUACULTURE

Department of Ichthyology, Biological Faculty, Moscow State University,
119899 Moscow, Russia

E-mail: pavlov@DPavlov.home.bio.msu.ru

Institute of Marine Research, Flødevigen Marine Research Station,
N-4817 His, Norway
)370-59000 Fax: (47)370-59001
E-mail: moksness@imr.no

The species of the genus *Anarhichas*
for cold-water aquaculture in northern Europe and in Atlantic Canada due to their high-quality meat, fast growth in captivity, and features of the life history.

approximately 20 last years were to study all main aspects of the life history of common wolffish (*lupus*) in nature and captivity. The majority of

Research, Flødevigen Marine Research Station (Norway).

Results and Discussion

et al., 1993), wolffish release fertilized eggs 8–15 h after ovulation. Within this time, copulation between spawners and

protected by the male, apparently until hatching. Incubation period lasts from 4 to 10 months in different regions. Larvae, 20-24 mm long, hatch at an advanced

stage of development, begin to feed at hatching, and transit to benthic habitats after a comparatively short period (1–1.5 months) of pelagic life.

Artificial insemination of wolffish eggs *in vitro* is the key event in the cultivation of this species. Spermatozoa with ultrastructure similar to that in oviparous fishes are motile in undiluted ejaculate (Pavlov et al., 1997). Apparently due to low mean sperm concentration (300 x 10^6 spz ml^{-1}) and large egg diameter (4.3-6.4 mm), a long egg-sperm contact (up to 7 h) is required for successful fertilization of eggs, and the duration of this contact depends on the sperm-egg ratio (Fig. 1)

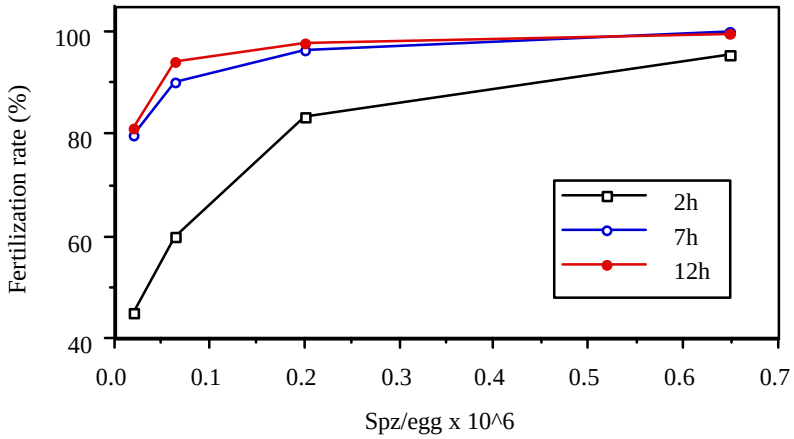


Figure 1. Fertilization rate of wolffish eggs inseminated with different sperm-egg ratios (number of spermatozoa per one egg) and different time of the contact between the eggs and sperm.

To increase the probability of the contact between gametes at a low ejaculate volume (about 2 ml from a male), a method of artificial insemination of eggs in the cylindrical vessels was worked out (Moksness and Pavlov, 1996) (Fig. 2).

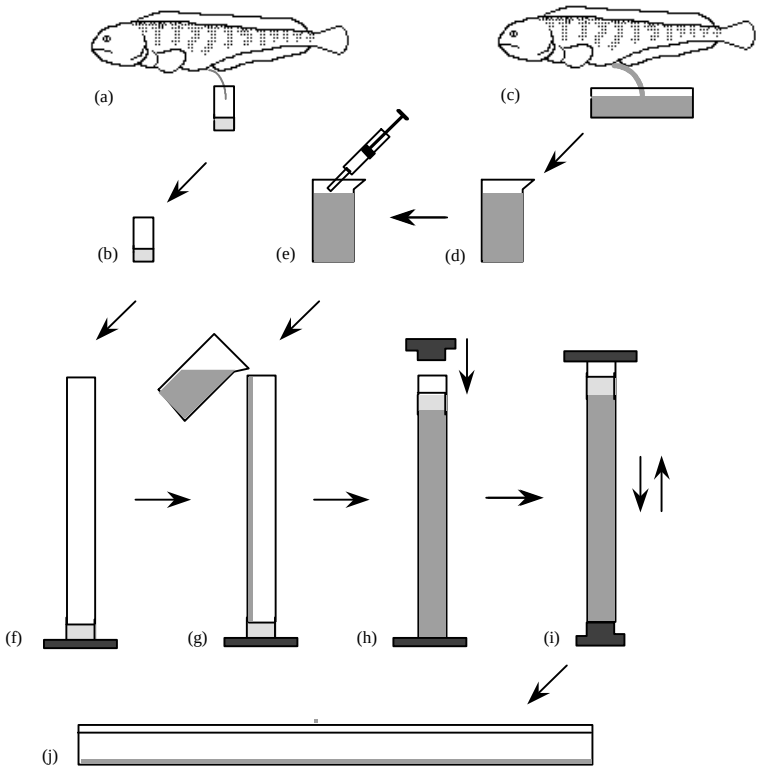


Figure 2. A scheme of the artificial insemination of wolffish eggs. (a) Collecting milt. (b) Milt storage (<10 h at 4°C). (c) Stripping eggs. (d) Egg storage (<12 h at 4°C). (e) Removal of some ovarian fluid to maximize subsequent sperm concentration. (f) Pouring sperm into cylindrical vessel. (g) Placing the eggs in the vessel. (h) Covering the upper part of the vessel. (i) Repeated mixing of eggs with sperm by inversions of the vessel kept at 2-7°C for 4-6 h (note that liquid ovarian fluid with the sperm appears at the upper part of the vessel while eggs sink to the bottom). (j) Distribution of the eggs on the bottom of large trays with stagnant sea water to prevent contact between eggs for at least 6 h to prevent their adhesiveness.

Free eggs obtained after preventing their adhesiveness were incubated in upwelling systems with periodic treatment by glutaraldehyde to prevent bacterial diseases. During most of the embryonic period, eggs can develop at temperatures ranging from -1 to 13°C. However, in larvae obtained from the eggs incubated at temperatures above 9°C, several axial skeleton anomalies were observed, including the absence of many rays in the dorsal and anal fins, fusion and destruction of some elements in the caudal skeleton (Pavlov and Moksness, 1997). A critical period determining the skeletal abnormalities (between 50% vascularization of the yolk sac and the beginning of formation of rays in the caudal and pectoral fins) was found. To reduce the egg incubation period and still obtain larvae without skeletal abnormalities, the incubation of eggs at lower temperatures during the critical period was suggested (Pavlov and Moksness, 1996).

Larvae were fed natural zooplankton and dry pellets. At age 1 month from hatching, maximum growth rate was observed at 11-14°C. In juveniles at age 9-12 months, the optimum water temperatures for growth and growth efficiency were 11°C and 9.7°C respectively (McCarthy et al., 1998). Common wolffish matured at a size >0.5 kg. Thus, the entire life cycle of this species was reproduced in captivity. At identical conditions the spotted wolffish (*A. minor*) will grow three times as fast as the common wolffish and reach approximately 5 kg in 2 years from start feeding (Moksness and Pavlov, 1996).

Acknowledgments

We would like to thank the Research Council of Norway for financial support.

References

Johannessen, T., Gjøsæter, J. and Moksness, E. 1993. Reproduction, spawning behaviour and captive breeding of the common wolffish *Anarhichas lupus* L. Aquaculture 115: 41–51.

McCarthy, I., Moksness, E. and Pavlov, D.A. 1998. The effect of temperature on the growth rate and growth efficiency of juvenile common wolffish (*Anarhichas lupus* L.). Aquacult. Intern. (in press).

Moksness, E. and Pavlov, D.A. 1996. Management by life cycle of wolffish, *lupus* L., a new species for cold-water aquaculture: a

Pavlov, ·P., Emel`yanova, N.G. and Moksness, E. 1997. Spermatozoon ultrastructure and sperm production in wolffish *Anarhichas lupus* Living Res. 10: 187–194.

Pavlov, D.A. and Moksness, E. 1996. Sensitive stages during embryonic *Anarhichas lupus* numbers of rays in unpaired fins and skeletal abnormalities. ICES J. Mar. Sci. 53: 731–740.

Anarhichas lupus (Pisces, Anarhichadidae), at different temperatures. 49: 401–416.

NEW DATA ON THE BIOLOGY OF THE PACIFIC SLEEPER SHARK, *SOMNIOSUS PACIFICUS* (SQUALIDAE) IN THE NORTHWESTERN PACIFIC OCEAN.

Alexei M. Orlov
Sergei I. Moiseev
Russian Federal Research Institute of Fisheries and Oceanography (VNIRO).
17 V. Krasnoselskaya, Moscow, 107140, Russia.
Phone: (095)264-94-54; Fax; (095)264-91-87;
E-mail: akrovnin@mx.iki.rssi.ru

Abstract

On the basis of 417 captures in the Bering Sea and 102 captures in the Pacific waters off the northern Kuril Islands and southeastern Kamchatka spatial distribution and bathymetry of Pacific sleeper shark, *Somniosus pacificus,* are considered. Their length frequencies and length-weight relations are given. The diet of this species in the western Bering Sea is described and feeding habits are analyzed.

Introduction

Pacific sleeper shark are common elasmobranch species on the shelf and continental slope in boreal and temperate waters of the North Pacific (Compagno 1984). In recent times this shark have a slight commercially importance in California waters (Walford *1935).* Japanese fishermen taken Pacific sleeper shark in the North Pacific Ocean in insignificant number (Zolotova 1978). Now this shark does not support a fishery (Osipov 1969; Compagno 1990). This species is a least studied. Most part of published data considered their descriptive characters and geographic distribution. Only several pa~ers included an information about stomach content of Pacific sleeper shark caught mainly in American coastal waters. The main purpose of this paper is to consider the features of spatial and vertical distribution of Pacific sleeper shark in the western Bering Sea and in the Pacific waters off the northern Kuril Islands and southeastern Kamchatka, to describe 'their size composition and length-

weight relationships in the areas considered, and to analyze the diet and feeding habits in the western Bering Sea.

Material and Methods

The data were sampled during several marine expeditions aboard Japanese trawlers in the Pacific waters off the northern Kuril Islands and southeastern Kamchatka during 1992-1997 and in the western Bering Sea during 1995-i 997 by Russian scientists.

Biological investigations were conducted according to Myagkov (1982). Specimens were measured from snout to the edge of upper lobe of caudal fin. Individuals with weight less than 100 kg were weighted using various spring-balance (20, 50, 100 kg). Larger fishes were cut to sections and then weighted.

The index of relative importance (WI) for each prey item was calculated according to Pinkas et al., 1971 as follow:

$$IRI=(\%N+\%W)\%FO,$$

where:

% N - percent of prey number;
% W - percent of prey weight;
% FO - percent of frequency of occurrence.

Results and Discussion

Pacific sleeper shark are an endemic of the northern Pacific Ocean (Roedel and Ripley, 1950; Pinchuk, 1968, 1972; Parin, 1971). This species are distributed from southern Shikoku (Japan) and Baja California (Mexico) to southern Chukchi Sea including northern Sea of Japan, Sea of Okhotsk, Bering Sea, Gulf of Alaska and waters off British Columbia, Oregon and Washington (Tanaka, 1935; Walford, 1935; Lindberg and Legeza, 1959; Clemens and Wilby, 1961; Gotshall ans 30w, 1965; Kato et al., 1967; Miller and Lea, 1972; Quast and Hall, 1972; Hart, 1973; Dolganov, 1983; Masuda et al., 1984; Compagno, 1984). Pacific sleeper shark was caught in bottom trawlings within the whole investigated area (Fig. 1). In the Bering Sea they occurred from 168E

(Olyutorsky Bay) to 178W, where catches contained mostly 1-10 individuals per trawling, but larger catches with over 25 specimens per trawling were noted between 1760 and 1780 E. In the Pacific waters off the northern Kuril Islands and southeastern Kamchatka Pacific sleeper shark were less abundant. All catches were represented only by single records.

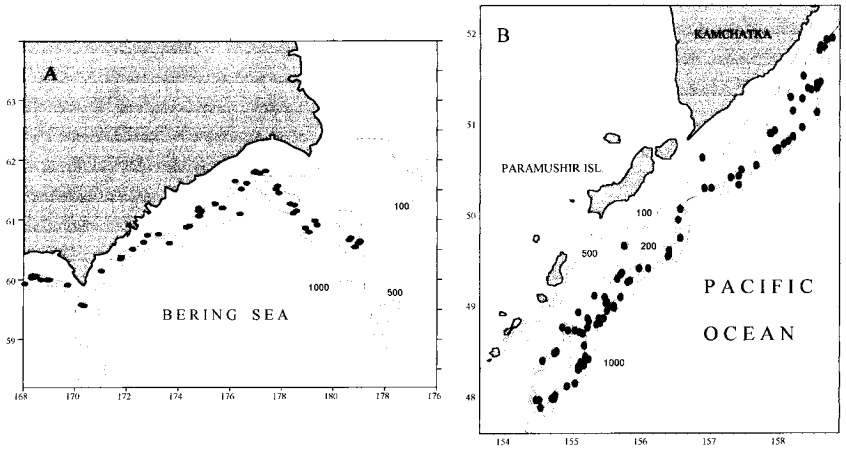


Figure 1. Maps of the study areas, showing trawl stations (dark dots) at which Pacific sleeper shark were caught during 1992-1997 (numbers are isobaths). A – western Bering Sea, B – Pacific waters off the northern Kuril Islands and southeastern Kamchatka.

Pacific sleeper shark inhabit shallower water in the northern areas where they may occurred near surface. In the southern areas they lower into the deep waters (Compagno, 1984). We found some distinctions in vertical distribution from described above. Depth ranges of occurrence of shark considered in bottom trawlings were 178-675 m (mean 457 m) in the western Bering Sea, and 85-717 m (mean 451 m) off the Kurils and Kamchatka. In both areas Pacific sleeper shark had two peaks in relative abundance (Fig.2). They noted for 400-450 m and 600-700 m in the western Bering Sea (28.3% and 51.7%, respectively), and for 250-300 m and 450-500 m off the Kurils and Kamchatka (17.8% and 38.6%, respectively). They are some seasonal variations in vertical distribntion of species considered. Thus, average depth of Pacific sleeper shark records off

Kurils and Kamchatka was 392.7 m in spring, 543.4 m in summer, 447.5 m in autumn, and 328.9 m in winter while in western Bering Sea seasonal variations in vertical distribution were not pronounced that may connected with more permanent environment of the Bering Sea slope region.

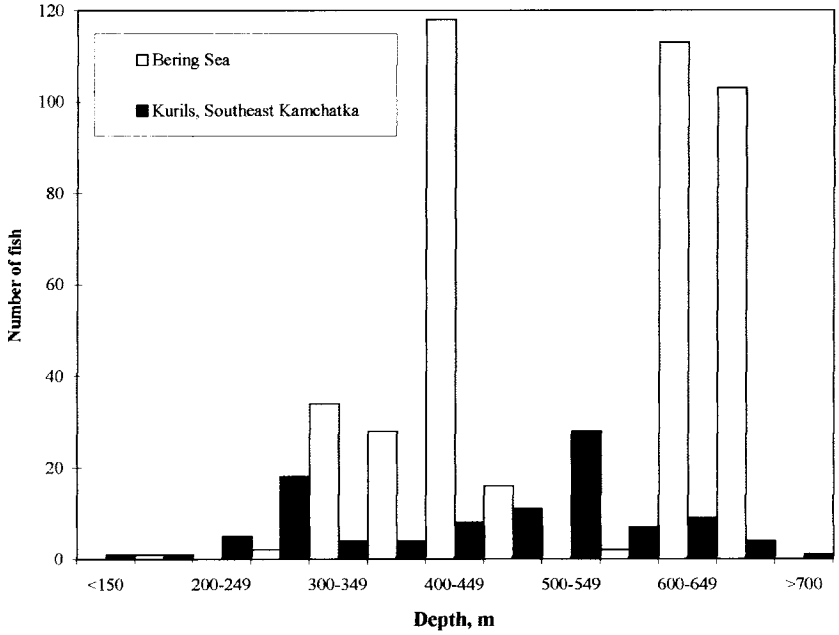


Figure 2. Bathymetric distribution of Pacific sleeper shark in the areas of the northwestern Pacific ocean in 1992-1997.

The common length of Pacific sleeper shark is *2.5-3* m, specimens with length about $ m are very rare (Soldatov and Lindberg, 1930). The maximum known length is 430 cm (Compagno, 1984). Hart (1973) as the limiting size of species considered noted length of 7.6 m. Compagno (1984) also reported that individuals with length over 7 m were photographed in deep waters. The size frequency distribution for Pacific sleeper shark showed that in the western Bering Sea this species attain a much greater size than off the Kurils and Kamchatka (Fig.3).

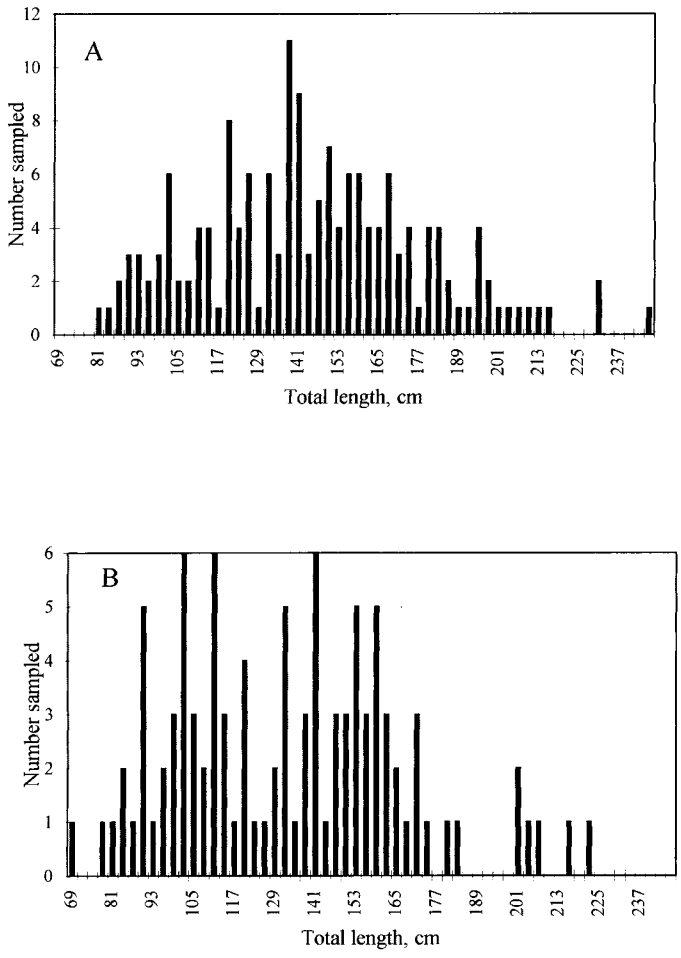


Figure 3. Length frequency distribution of Pacific sleeper shark in the areas of the northwestern Pacific Ocean in 1992-1997. A- western Bering Sea (total 162 fish examined), B – Pacific waters off the northern Kuril Islands and southeastern Kamchatka (total 99 fish examined).

This shark was caught in the first area with length 83-230 cm (mean 142.23 cm) and in the second one with length 36-220 cm (mean 131.7~ cm). The modal size classes were 138 cm Th for western Bering Sea, and 102, ill, and 141 Th for Pacific waters off the northern Kurils and southeastern Kamchatka. The total weight of shark varied from 4.7 to 122 kg (mean 28.5 kg) in the first area, and from 1.8 to 100 kg (mean 28.1 kg) in the second one. Equations of weight-length relations for both areas were fitted as:

wt (kg) = 6.959 length (cm) 3.047 (western Bering Sea, 75 specimens, R=0.981)

wt (kg) = 3.132 length (cm) 3.245 (Kurils and Kamchatka, 50 specimens, R=0.943)

The recently data on diet of Pacific sleeper shark are very limited. The prey known from their stomachs are tanner crab *Chionoecetes bairdi,* hairy triton *Fusitriton oregonensis,* octopuses *Octopus* sp., miscellaneous squids, rex sole *Glyptocephalus zachirus,* dover sole *Microstomus pacficus,* chinook salmon *Oncorhynchus tschawytscha,* Pacific halibut *Hippoglossus stenolepis,* shortspine thornyhead *Sebastolobus alascanus,* harbor seal *Phoca vitulina,* and carrion (Phillips, 1953; Bright, 1959; Hart, 1973; Compagno, 1984). Our investigation showed that Pacific sleeper shark in the western Bering Sea are predator, which feed mostly on cephalopods and fish (Table). The most frequent and numerous prey were red squid, *Berryteuthis magister,* which was found in shark stomachs mostly as beaks and digested remains. Weight percentage of these prey category was only on the fifth place, but red squid occupy basic position according to the value of WI. The second and third place belongs to offal (WI=145.3), and giant grenadier, *Albatrossia pectoral/s* (RI=145.2). The considerable role in the diet played also walieye pollock, *Theragra chalcogranima,* popeye grenadier, *Coryphaenoides cinereus,* chum salmon, *Oncorhynchus keta,* Kamchatka flounder, *Atherestes evennanni,* and unidentified fish.

The dietary variations in various size groups were detected. Upon the whole, the increase in size was characteristic of decline in consumption of cephalopods and rise of that of fish (fig. 4). Red squid were the most important prey of size group with length under 139 cm. Sharks with length of 120-159 cm fed mainly on grenadiers. In 160-199 cm size groups the consumption of salmons was significant. Offal was eaten by Pacific sleeper shark in all size groups in almost the same number.

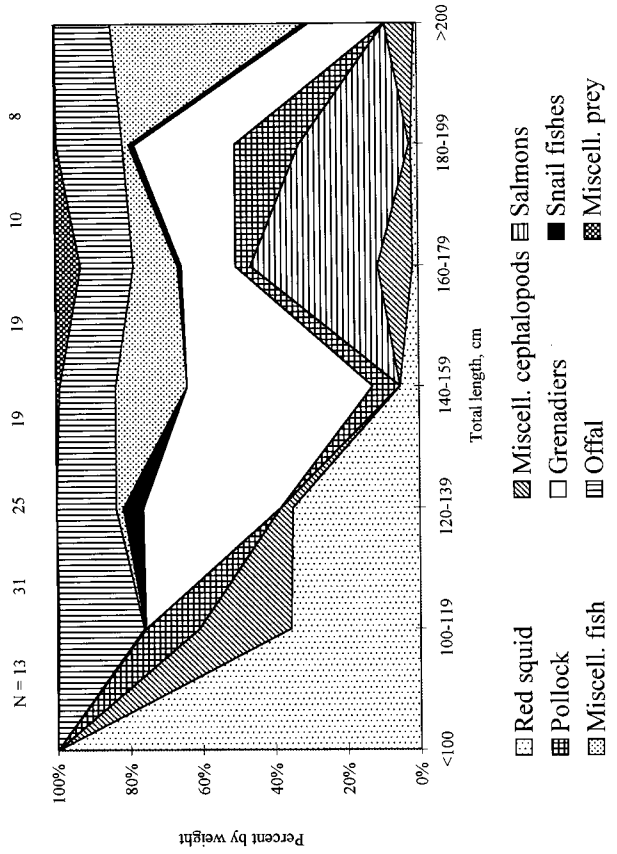


Figure 4. The diet of Pacific sleeper shark by percent weight in the western

number of stomachs that contained food in each size group).

References

The occurrence and food of the sleeper shark, *Somniosus* in a central Alaska Bay. Copeia, No.1, P.76-77.

Clemens W. A., and 0. V. Wilby. 1961. Fishes of the
Bull. Fish. Res. Board Can. No.68, P.1-443 p.

Sharks of the world. FAO Fisheries Synopsis, Vol.4,

Compagno, L.J.V. 1990. Shark exploitation and conservation. P.391414 In: H.L.

resources: advances in the biology, ecology, systematics, and the status of the fisheries. U.S. Dept. Commer

Dolganov, V.N. 1983. Guide to the diagnostic characters of cartilaginous fishes from the Far East Sea of the U.S.S.R. and neighboring waters.
Vladivostok, 92 p. (In Russian).

Gotshall D.W., and T. Jow. 1965. Sleeper sharks (Somniosus pacificus) off

No.4, 294-298.

Hart, J.L. 1973. Res. Board Can., No.180,
P.1-740.

Hawaiian sharks. U.S. Dept. Interior, Fish Wildlife Serv., Bur.

Lindberg CU., and M.I. Legeza. 1959. Fishes of the Sea of Japan and adjacent parts of the Sea of Okhotsk and Yellow Sea. Part 1.
Akademii Nauk SSSR, 208 p.

Masuda, H., Amaoka, K., Araga, C., Uyeno, T., and T. Yoshino (Eds.). 1984. 3 Vols. Tokai Univ. Press.
P.22+437, 20+448, 370 pls.

Lea. 1972. Guide to the coastal marine fishes of California. Fish. Bull.

Myagkov, N.A. 1982. Methodical guide to data sampling and jnitial study of Moscow, VNIRO, 28 p. (In Russian).

Osipov, V.0.1969. The sharks: visual aid for fishermen. Vladivostok, Dal'nevostochnoye Knizl:noye Izdatel'stvo, 33 p. (In Russian).

Parin N.V. 1971. Order Squaliformes. P.49-52 In: T.S. Rass (Ed.) Wildlife. Vol.4, Part 1 (Fishes). Moscow, Prosveshcheniye.

Phillips J.B. 1953. Sleeper shark, *Somniosus pacficus*, caught off Fort Bragg, California. Calif Fish Game, Vol.39, No.1, P.147-149.

Pinchuk V.1.1968. Field guide to sharks. Moscow, VNIRO, 44 p. (In Russian).

Pinchuk V.1. 1972. Field guide to sharks of the world. Moscow, Pishchevaya Promyshlennost', 240 p. (In Russian).

Pinkas L., M.S. Oliphant, and I.L.K. Iverson. 1971. Food habits of albacore, bluefin tuna, and bonito in California waters. Fish Bull. Calif Fish Game, *No.152,* P.1-lOS.

Quast, J.C., and E. L. Hall. 1972. List of fishes of Alaska and adjacent waters with a guide to some of their literature. U.S. Dep. Commer., NOAA Tech. Rep. NMFS SSRF- 658, 47 p.

Roedel P.M., and W.E. Ripley. 1950. California sharks and rays. Fish Bull., Calif Fish Game, No.75, P.1-88.

Soldatov V.K., and G.U, Lindberg. 1930. Review of fishes of the Far East seas. Izv. TINRO, Vol.5, P.1-576. (In Russian).

Tanaka S. 1935. Figures and descriptions of the fishes of Japan including Riukiu Islands, Bolin Islands, Formosa, Kurile Islands, Korea, and Southern Sakhalin. Tokyo, Kazamashobo, Vol. I-XXX, 557 p.

Walford, L.A. 1935. The sharks and rays of California. Fish. Bull., Calif Fish Game, No.45, P.1-66.

Zolotova, Z.K. 1978. Shark fishery perspectives in the World Ocean. Obz. Inform. CNIIThIRH, Ser.1, No.6, P.1-30. (In Russian).

Table 1 - --Prey items (expressed in frequency of occurrence, number, and weight) and its relative importance if diet of Pacific sleeper shark, *Somniosus pacificus*, collected in the western Bering Sea in 1997. (IRI Index of relative importance).

Prey name	% Fr	Number		Total	weigh	'RI
-	. occur.	Item	%	g	%	
Spongia (sponge)	0.79	1	0.08	60.0	0.13	0.17
Anonyx sp. (amphipod)	2.36	6	0.47	30.0	0.06	1.27
Gastropoda (snail)	0.79	1	0.08	40.0	0.09	0.13
Berryteuthis magister (red squid)	77.95	1169	93.34	4535.7	9.85	8034.3
Moroteuthis robusta (giant squid)	*1.57*	2	0.16	1260.0	2.74	4.55
Belonella borealis (squid)	0.79	1	0.08	40.0	0.09	0.13
Octopoda (octopus)	2.36	3	0.24	1470.0	3.19	8.09
Clupeapallasi (Pacific herring)	0.79	1	0.08	100.0	0.22	0.24
Oncorhynchus keta (chum salmon)	2.36	3	0.24	6320.0	13.72	32.95
Oncorhynchus sp. (salmon)	0.79	1	0.08	*45.0*	0.10	0.14
Stenobrachius leucopsarus (northern lampfish)	0.79	1	0.08	8.0	0.02	0.08
Theragra chalcogramma (walleye pollock)	3.94	8	0.63	2730.0	5.93	25.86
Albairossia pectoralis (giant grenadier)	7.87	10	0.79	8130.0	17.65	145.20
Coryphaenoides cinereus (popeye grenadier)	7.87	24	1.90	3690.0	8.01	78.07
Lycodes soldatovi (celpout)	0.79	1	0.08	1000.0	2.17	1.78
Malacocottus zonurus (darkfin sculpin)	0.79	1	0.08	95.0	0.21	0.23
Careproctusfrrcellus (forlttail snailfish)	*1.57*	*2*	*0.16*	*275.0*	*0.60*	*1.19*
Elassodiscus tremebundus (snailfish)	1.57	2	0.16	470.0	1.02	*1.85*
Atherestes evermanni (Kamchatka flounder)	2.36	4	0.32	7000.0	15.20	36.63
Unidentified fish and flsh remains	10.24	12	0.95	*652.0*	1.42	24.37
Pinniped remains (carrion?)	0.79	1	0.08	760	*1.65*	1.37
Offal (fishery discards)	8.66	11	0.87	7330.0	15.91	145.3
Unidentified organic materials	0.79	1	0.08	17.0	0.04	0.09

Total prey number	1266
Total prey weight	46056.7
Number of stomachs with food	125
Number of empty stomachs	23

MORPHOLOGICAL VARIATION

AND NEW SYSTEMATIC STATUS

OF "*COBITIS TAENIA* L." COMPLEX

FROM CROATIAN RIVERS

Daniela Schneider
[1]Faculty of Science, Department of Zoology, University of Zagreb
Rooseveltov trg 6, 10 000 Zagreb, Croatia
Tel:4+385- 1-4826-263, *++385-1* -4326-264, Fax: *++385-1* -4326-263
E-mail: Daniela.Schneider@public.srce.hr

M. Mrakovcic[1], M. Kerovec[1], Perica Mustafic' and Zlatko Mihaijevic'

Introduction

The loach subspecies from the Croatia were shortly described from the Karaman 1928. More detailed analyses have never been made. In this work the *Cobitis* populations were described from the Danube watershed (Drava) and from the Adriatic river basin (Zrmanja, Cetina and Nerewa). The aim of this work was to redefine the taxonomic status of these populations.

Materials and Methods

The loaches were caught by the electro-fishing aggregate and preserved in the 4% formalin. To determine the taxonomic status the Croatian loaches were analyzed on the morphometric (truss network and classical measures) and the phenotypic features (canestrini scale, suborbital spine, color pattern and shape of mouth).

Results

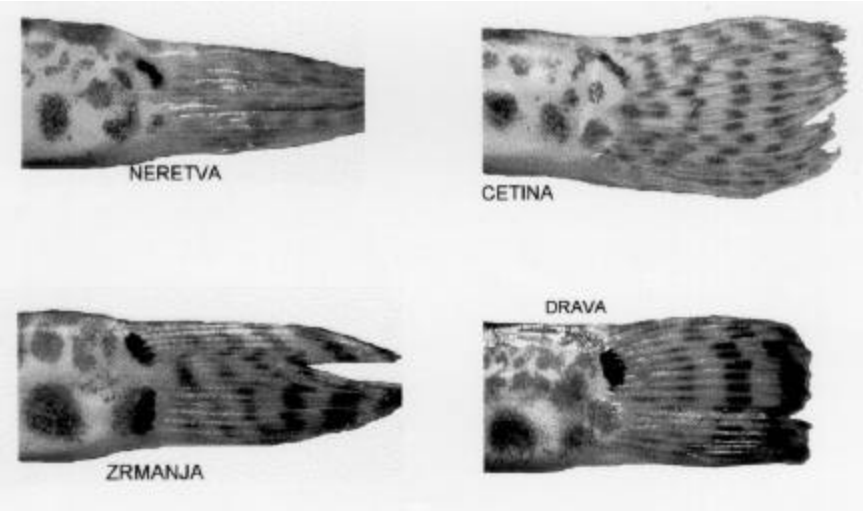
The morphometric results showed bimodal distribution for the males and females of the Zrmanja and also for the females of the Cetina. The tAest for the

classical method showed the minimum taxonomic differences at the level p<0.0l between the both male and females of the Neretva and the Drava populations and between the Neretva and the Cetina populations. The tAest for the truss network morphometry was made separately for the males and females. Toward supposed taxonornic differences the least similar were the Cetina and the Zrmanja populations and the Zrmanja and the Drava populations. The very similar were the Neretva and the Cetina population. The strange thing was that between females of the Nerewa and the Drava were only 4 differences, one at the level p<0.01, while between the males of the same populations all the measures were different at the level p<0.Ol. Also no difference was found for the females of the Cetina and the Drava, while the males were different ahnost in the all measures at the level p<O.0l. In the phenotypic characters the axe shaped canestrini scale has the Nerewa and the Drava populations. This scale in the Cetina population is more width than high and in the Zrmanja population the shape is oval. The Nerewa and the Drava population have the short and thick barbells, which are thicker in the Neretva loaches. The Cetina population has the longest barbells. The Zrmanja population has very thin barbells and also they are pigmented (Figure 1).

The shape of suborbital spine is different between the all populations, especially in the Neretva population. Almost every population has the 3 type of the color pattern, but the Cetina has four. For the Adriatic populations the spot at the base of the caudal fin has an awry position. For the Neretva population this spot is dark brown and the kidney shaped and beneath this one is hardly seen one little oval spot. In the Cetina population the spot is brown and narrow. The Zrmanja population is the specific one because of the existence of two well visible spots, one oval and black and the second brown spot beneath this first. The Drava population has the very visible oval black and shiny spot (Figure 2).

Discussion

C. tenia is characterized with the great variability of the plastic characters especially for the color pattern (]3anarescu 1964). The former investigations of the loaches indicated that the *"Cob itis taenia* L.[19] represents a common group, which consists of the several different subspecies or species. The results obtained in this work point out the possibility of the existence more than one population in the Adriatic rivers. This is indicated with the bimodal distribution in the Cetina and the Zrmanja populations, and with the great morphometric similarity in the Nerewa and Cetina females with the Drava females. Maybe the population from the Drava (')anube watershed) came in the contact with the Cetina and the Neretva loaches as it has happened in the Pleistocene during the progressive cooling climate &conomidis & Nalbant 1996). Is it also possible that the females from the Nerewa and the Cetina are hybrids with the populations from the Danube watershed while the males have their original origin. The investigations on the loaches from the Ukinski lug (central Bosnia) (SofradzUa & Berberovic 1978) has showed that all the females examined were triploid (3n=75) while the males were diploid *(2n=50).* In spite of this all the phenotypic features both in the males and the females indicate that these four populations are completely different from the each other, especially this difference is in the spots on the base of the caudal fin, then the suborbital spine shapes and the shapes of the barbells. With these different characters the populations could not be mixed up and could not be the subspecies as Karaman 1928. considered them.



References

Banarescu, P.1964. Fauna republicii popul&e romine: Pisces-Osteichthyes. Editura academiei republicii popolare Romine. Bucuresti. Vol.12, *522 p.*

Economidis, P.S. & Nalbant, T. 1996. A study of the genera *Cobitis* and Sabanejewia (Pisces, Cobitidae) of Greece, with description of six new taxa. Trav. Mus. natl. Hist. nat. "Grigore Antipa", Bucuresti. 36: 295-347.

Karaman, S. 1929. Prilozi ihtiologiji Jugoslavije. Glasnik Skopskog naucnog drustva. hi 6. Skoplie. 147-176.

Sofradzija, A. & Berberovic, Lj. 1978. Diploidno-triploidni seksualni dimorfizam u vUuna *(Cobitis taenia taenia* **L. Cobitidae,** Pisces). Genetika, Vol.10, No.3, Beograd. 389-397.