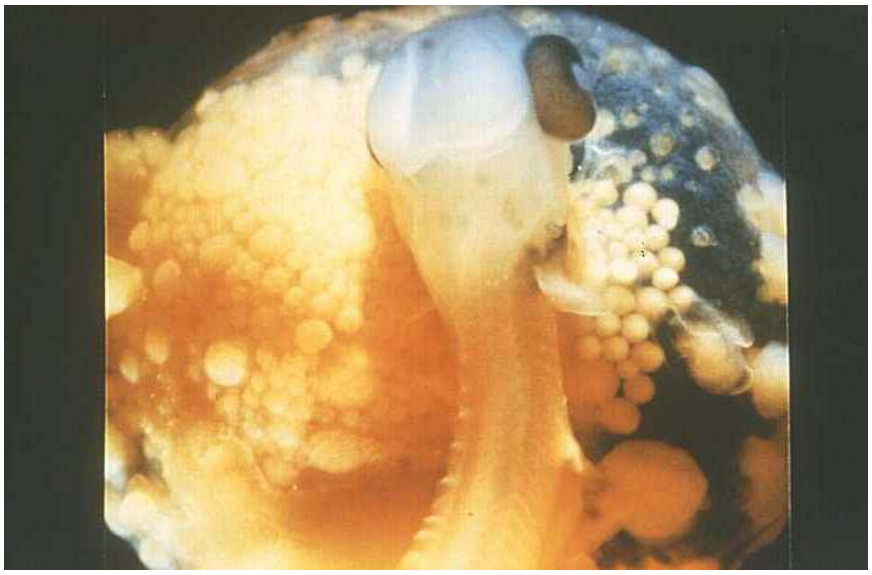
Incubation of Fish:

Biology and Techniques



John Jensen
Craig Clarke
Don M[ac]Kinlay

International Congress on the Biology of Fish
University of British Columbia, Vancouver, CANADA

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SYMPOSIUM PROCEEDINGS

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PREFACE

The early life history phase of fish continues to present new topics of interest for researchers. This symposium has gathered together research findings on the biology and techniques of egg incubation. The papers presented include research on factors that influence egg and sperm quality, fertilization, and embryonic development and survival. With the information presented, researchers and fish culturists will have a better understanding of key factors that influence gamete quality and embryo development and survival. Finally, several papers also present tools or techniques for improving egg growth and survival.

Symposium Organizers:

John Jensen, Craig Clarke – Pacific Biological Station, DFO

Don MacKinlay – Habitat & Enhancement Branch, DFO

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The sponsors included:

- Fisheries and Oceans Canada (DFO)
- US Department of Agriculture
- US Geological Service
- University of British Columbia Fisheries Centre
- National Research Council Institute for Marine Biosciences
- Vancouver Aquarium Marine Science Centre

The main organizers of the Congress, on behalf of the Physiology Section of the American Fisheries Society, were Don MacKinlay of DFO (overall chair, local arrangements, program and proceedings) and Rosemary Pura of UBC Conferences and Accommodation (facility arrangements, registration and housing). Thanks to Karin Howard for assistance with Proceedings editing and word-processing; to Anne Martin for assistance with the web pages; and to Cammi MacKinlay for assistance with social events.

I would like to extend a sincere 'thank you' to the many organizers and contributors who took the time to prepare a written submission for these proceedings. Your efforts are very much appreciated.

Don MacKinlay
Congress Chair

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REPRODUCTIVE BIOLOGY AND EARLY LIFE HISTORY OF YELLOWTAIL KINGFISH SERIOLA *LALANDI LALANDI*

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EXTENDED ABSTRACT ONLY- DO NOT CITE

The National Institute of Water and Atmospheric Research (NIWA) Ltd and various collaborators are conducting underpinning research to develop techniques for yellowtail kingfish (*Seriola lalandi lalandi*) aquaculture in New Zealand. Among other attributes, kingfish are being targeted as an aquaculture species due their fast growth, excellent flesh quality and significant market opportunities internationally. To date we have established a breeding population of captive kingfish which spawn spontaneously and provide eggs on a daily basis during the spawning season. The resulting eggs have been reared through larval and juvenile stages and successfully ongrown to 3 kg in 12 months. While we have moved forward quickly in establishing basic rearing techniques and information of kingfish biology, we have also identified the following technological barriers to commercial kingfish aquaculture: sub optimal egg quality, high larval mortality soon after first feeding and jaw deformities.

To establish egg collection techniques and ultimately refine breeding control, we collected information on the reproductive biology and endocrinology of wild populations. Our research indicated that kingfish have multiple group synchronous gamete development; spawn in spring/summer (October to January); and first reach reproductive maturity at 750 and 775 mm FL for males and females respectively (Poortenaar et al., 2001). In females, blood plasma concentrations of testosterone and estradiol peaked during vitellogenesis and concentrations of 17α20β-dihydroxy-4-pregnen-3-one (17,20βP) were elevated during final oocyte maturation. In males, plasma concentrations of 17,20βP did not change with testis development, however, concentrations of testosterone and 11-ketotestosterone were elevated in partially and fully spermiated males (Poortenaar et al., 2001). Reproductive profiles from natural cycles provide:

a benchmark to gauge reproductive health in broodstock; and a basis for phototherm control.

Our routine assessments of egg production cycles and egg quality indicate that egg viability is on average 35%, and a significant proportion of viable eggs have blastomere deformities. Possible causes of poor egg quality include stress and sub optimal nutrition. We assessed plasma: cortisol; lactate; and pH and blood: glucose and hematocrit in response to capture, transport, confinement and handling. While stress responses to these routine husbandry practices were similar to other marine teleosts, we could not confidently determine the resting status of our captive broodstock, because the logistics involved in sampling the fish induced a stress response.

In the absence of running costly feed trials, we formulated a broodstock diet based on information on related species, and will continue to refine the diet as biochemical and physiological data on kingfish becomes available. For example, in future studies we will examine egg and larval energetics and metabolism, and we are currently assessing protein and fatty acid profiles during egg and larval development. This information will also contribute to the development of a larval feeding regime, intended to improve larval survival. By staining for bone and cartilage, we hope to identify the onset and nature of jaw deformities (Figure 1) as a basis for confirming the cause.

Recent larval rearing trials indicate that kingfish larvae are much more prone to bacterial pathology compared to our experience with other species. The use of antibiotics enhanced survival, most likely by limiting bacterial abundance and diversity introduced with food organisms. Future rearing protocols will include greenwater culture, antibiotic, and possibly probiotic procedures specifically related to the optimum feeding strategy. The influence of these procedures on gut flora and digestive enzymology will be considered.

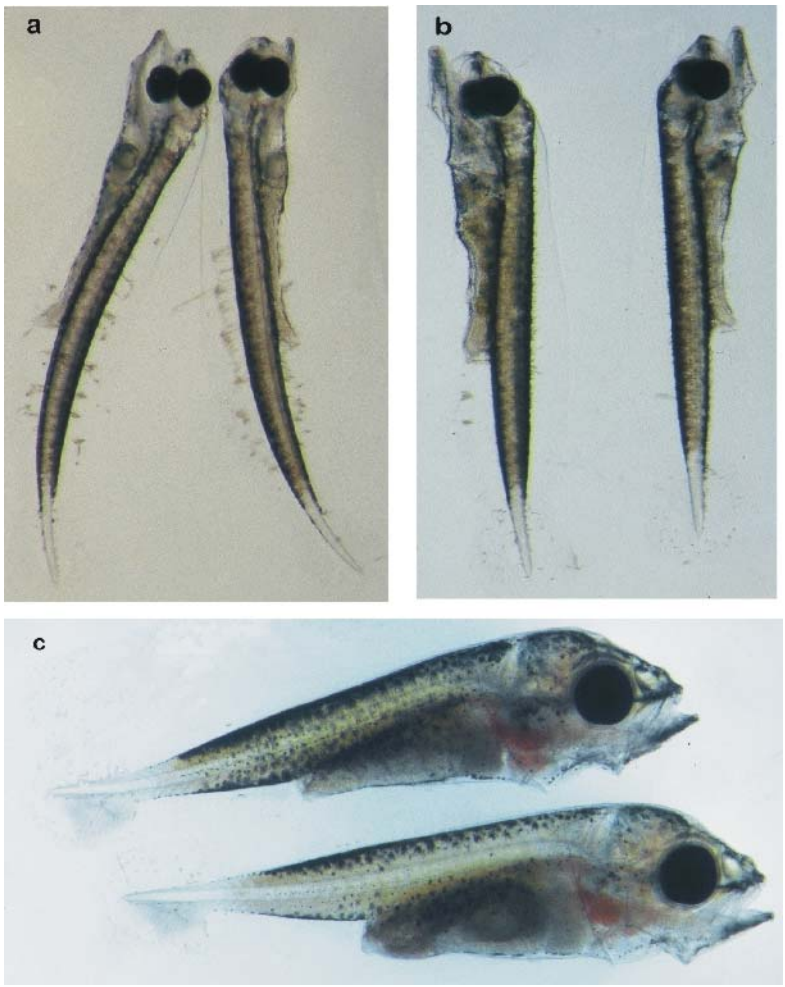

Figure 1. Examples of jaw deformities in kingfish larvae at 4, 8 and 15 days post hatch for a, b and c respectively.

Additional research is also being conducted on the sensory ontogeny of kingfish larvae, with particular reference to the visual and lateral line sensory systems. Of primary interest is how light intensity and spectral sensitivity influence feeding success. Results to date indicate that superficial neuromasts of the lateral line system are present 2 days post hatch (DPH), and most likely function in the detection of predators; provision of input into the M-cell escape response; and prey capture if visual conditions are poor. Retinal pigmentation is complete 4 DPH and co-incides with depletion of yolk reserves, indicating 'sensory readiness' for the switch to exogenous feeding.

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Acknowledgements

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INCUBATION OF BENTHIC EGG MASSES

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Pelagic eggs must be incubated with water flows that maximize respiratory exchange across the chorions of all individual eggs. For benthic egg masses, in which the eggs adhere to each other and in some cases to the substrate as well, water flows may be restricted by points of chorion attachment as well as by egg mass morphology and attachment site geometry. Optimal egg incubation requires that flows pass individual eggs equally.

For Pacific herring (*Clupea pallasi*) it has been shown that poor flows which cause egg mortality will result in chronic developmental retardation among those larvae which do survive to hatch from "overspawned" egg clusters (Marliave, 1995). Herring tend to prefer spawning substrates that minimize clumping of spawned eggs, which in turn maximizes percent hatch as well as viability during larval stages.

With lingcod (*Ophiodon elongatus*) the territorial male chooses a boulder or crevice which tends to deflect tidal currents or prevailing wave surge in such a way that flows through the egg mass are maximized. Giorgi (1981) demonstrated that nesting sites with inadequate flows for incubation result in high levels of egg mortality. In a laboratory situation, creation of a venturi effect is required, by means of placing baffles against the egg mass, so that currents flow through the egg mass rather than around it.

Another aspect of incubating benthic egg masses involves protecting the embryos from mechanical or other stimulation near the time of hatching, so that hatching enzymes are secreted gradually, as the embryos approach optimal development for hatching. Post-hatch survival data for lingcod which hatched from undisturbed embryos showed over 60% survival at 5-d post-hatch versus under 20% survival among larvae from a stimulated hatch (Marliave, et al., 1987). Forced hatches tend to show very high mortality prior to yolk resorption.

The wolf-eel (*Anarrhichthys ocellatus*) also spawns under boulders or in crevices, but the spherical egg mass does not adhere to the substrate. Parents wrap their bodies around the eggs to hold them in place. In a laboratory situation, the same type of baffle system to create a venturi effect can be used to optimize egg incubation. The techniques of venturi incubation and undisturbed hatch, together with a newly available frozen copepod food, have resulted in the licensing of wolf-eels for commercial growout in the BC aquaculture industry.

Incubation of benthic egg masses should yield a hatch of virtually all eggs and the larvae should show high survival through yolk resorption in order to have reasonable expectation of robust growth and survival through to adult size. It is not fair to say that, if they hatch, they hatch; rather, it must be expected that suboptimal egg incubation will result in chronic detrimental impacts on feeding and swimming behavior, as well as on growth and survival.

A point-source of flow from a pipe in a tank, even at high velocity, cannot equal the total energy of a uniform tidal flow in nature. Furthermore, turbulent, point-source inflows will tend to cause current around an egg mass rather than through it. For these reasons, it is necessary to restrict flow direction with baffles or inside a pipe, so that seawater is forced through the interstitial spaces between the eggs within an egg mass. This incubation system is best set up within the intended tank for larval rearing, in order that the egg mass can be allowed to reach hatching without any mechanical disturbance.

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OPTIMIZING SALMON

INCUBATION OPERATIONS

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Introduction

Incubation is the process of turning a bag of chemicals into a living organism. This "miracle of life" transformation, the most profound event in the history of the universe, occurs every time an egg is successfully fertilized. While over 3 billion years of evolution has done a pretty good job of making the process fairly robust, fish culturists can still play an important role in giving each individual miracle its best shot at success.

Some points should be kept in mind when thinking of how to optimize conditions for incubation:

First, the process of incubation evolved with a number of assumptions about the environment in which it would occur, and is adapted to work well in that environment. For salmonids, this usually means the environment of fairly pristine mountain streams, and for Pacific salmon, it means cold, clear, high-oxygen, high-flow gravel beds. This is usually the kind of environment we try to mimic in fish culture facilities, using ultra-clean water and avoiding temperature extremes.

Second, this 'typical' environment is not necessarily 'ideal' for the incubation process. The reason that salmonids (a marine fish) sought out freshwater streams in which to lay their eggs probably had more to do with avoiding the abundance of predators in the ocean than that streams are a perfect incubation environment. (it is in fact the inhospitable-to-life nature of mountain streams, with their lack of nutrients, shifting substrate and cold

temperatures, that is appealing to salmonids. Neither predators nor pathogens can survive in these locations during the long periods when salmon eggs are not available.

Third, optimization is an arrow that needs a target before deciding which way to aim the bow. Usual targets are perfect survival or maximum fry weight, but targets such as highest disease resistance or shortest swim-up timing might require aiming in a different direction. The fish culturist does not have to blindly follow nature's lead but has many options to manipulate incubation outcomes to serve whichever 'optimal' target is selected.

Fourth, while the water supply at every hatchery is undoubtably not 'optimal' for incubation, embryonic development is so efficient and low-key that it has little impact on the water used for it. Therefore, water supplies can be fairly inexpensively re-engineered to be more 'optimal' than what they were originally, using recirculation technology.

The incubation process starts off with the production of the eggs and sperm in the bodies of the broodstock, includes the stripping and fertilization of the eggs and their placement and care in incubation containers, and ends when the fry have swum up and started to feed. Each of these steps can be improved by improving the environment (physical and chemical) in which they occur, by removing deleterious effects (like pathogens or toxins) and by providing stimuli to accelerate (or decelerate) development.

Adult Holding

The role of the adult in incubation is to furnish the process with good-quality eggs and sperm, or more correctly, to take the best care of itself so that good quality eggs and sperm can be produced.. The role of the fish culturist is to take the best care of the adults so that they are in fit condition to produce good quality eggs and sperm.

The processes of egg and sperm production within maturing adults is a long and complex sequence of precisely timed and executed events. These events are signalled and triggered by a cascade of hormone releases that start before the germinal cells begin to undergo meiosis to produce gametes and are still going on while eggs and sperm are being shed from the adults' bodies.

We have to understand that the adult salmon has other things on its mind, in addition to procreation, while it is creating eggs or sperm, some of which, like

survival, take precedence. The amount of energy that an adult puts into gamete production is dependent on the amount of energy that it has to spare. The fish culturist should seek ways to minimize the requirement for energy use for other things by the adults, to maximize the effort available for gamete production.

Some guidelines are:

Provide an optimal environment:

- Temperature - cool enough to keep oxygen levels high, metabolic rates low and reduce invasion by pathogens, but warm enough to allow thorough gamete development.
- Light - low enough to reduce excitement.
- Space - room enough to move and avoid rubbing against each other and the container walls but not enough to establish territories.
- Water Flow - enough for sufficient oxygen supply and waste removal.
- Chemical properties: ionic content of the water should be sufficient to minimize leaching of essential ions needed for egg development.

Protect from pathogens:

- Minimize handling to avoid the abrasions, stress and trauma that can encourage infection.
- Maintain physical separation between broodstock and sources of pathogens or toxins (birds, rodents, wild fish, people).
- Inject or bath with antibiotics to control pathogen growth on adults being held.

Stimulate development

- Manipulate degree of isolation from each other to stimulate or delay maturation, depending on the preferred timing of spawning.
- Inject hormones (e.g. LHRH) to stimulate final maturation and synchronize timing of egg-takes.
- Provide nutrients in food or water that will aid in gamete development.

Fertilization

The first requirement of successful fertilization is good quality eggs and sperm, which is mainly determined by how the adults were treated. Once removed from the adults, the gametes may or may not have all that is required to produce a living embryo. The fish culturist has many options available to make the fertilization process as near perfect as the quality of the eggs and sperm will allow.

Some guidelines:

Provide an optimal environment

Ensure that water temperature, oxygen content, other dissolved gas pressures and light are suitable at every stage of the process. Test the egg's environment with an oxygen probe and thermometer every minute during a typical (or extreme) egg-take procedure to convince yourself that the eggs (and sperm) are well taken care of at all times.

Manipulate the ionic content of the water used for fertilization, washing and hardening, to ensure that there is enough sodium, calcium and other ions for proper fertilization (Brown and Lyman, 1981) and hardening (Li et al., 1989). Such a small amount of water is needed for these activities that almost every hatchery could benefit from using a prepared mix rather than just using the available water (Rieneits and Millard, 1987).

Use procedures that eliminate occurence of blood or broken eggs in the fertilization buckets. Use a bicarbonate soda wash (Wilcox et al, 1984) to dilute any cytoplasm (potassium) in the ovarian fluid, since it severely inhibits sperm activation (Morisawa et al., 1983).

Protect from pathogens

Use test kits to screen for known pathogens and eliminate carriers from the egg pool.

Select only those eggs that are at their peak of ripeness. Do not use eggs from over-ripe females with watery ovarian fluid, nor eggs that have had to be torn out of the skein. Test the fertilizability of eggs from different parts of the abdomen and taken from fish with different degrees of softness to determine the best time and procedure for extracting eggs.

Handle eggs and sperm carefully during extraction, storage and mixing to minimize physical damage.

Make sure that your wash water, fertilization water and hardening water are all sterile.

Stimulate development

Consider using an activator solution to stimulate or prolong sperm activity (Moccia and Munkittrick, 1987).

Manipulate the content of the hardening water content to see if you can provide essential nutrients (Ronnestad and Fyhn, 1993) or ions to the egg at the only time when it is taking in large amounts of external water. After hardening, the egg becomes quite impervious to movement of all but the smallest chemicals through the shell.

Egg and Alevin Incubation

Many of the fish culture procedures and criteria for incubation are determined by the containers in which the fertilized eggs are kept during the incubation period. Biologically, the choice of container should not make much difference to the fish, since every kind should provide the same kind of even, gentle flow that brings oxygen and removes wastes from the area around every egg. Care needs to be taken that a container or the way it is loaded does not crush or deform eggs.

The stages of development that an egg goes through after fertilization, from the combining of the sperm and egg haploid nuclei, through the first cell division and formation of the blastula, the gastrula, the neural fold, the eyes, etc. are incredibly complex (Velson, 1980) and are made up of, and controlled by, biochemical reactions that were pre-set, all ready to go, in the bag of chemicals (Hamor and Garside, 1977) that was produced by the female salmon (Brachet and Alexandre, 1986).

Provide an optimal environment

Ensure that water quality is kept at the highest standards, including oxygen, nitrogen and total gas pressures, ammonia, nitrite and carbon dioxide. Micro-environments within an incubation container can be very different from one another due to the pattern of water flow or stagnation within the incubation container.

Add ions to your process water if it is very soft or acid and soften the water if it is extremely hard (Gunn and Keller, 1980).

Keep the temperature within the metabolic limits of the fish (Weatherly and Gill, 1995), with the understanding that this is one area where the environment can be manipulated to suit your needs, since those of the fish are very plastic.

Minimize disturbance by keeping light and sound (vibration) levels very low.

Provide media for alevins to lean up against, reducing energy wasted in thrashing around.

Protect from pathogens

Thoroughly disinfect both the containers and the fertilized eggs at the beginning of incubation.

Start off with a clean water supply and make it even cleaner with disinfection.

Take every step possible to ensure a pathogen-free environment, including limiting work around and access to the incubation area.

Pick dead eggs out of the system as soon as possible. Pre-eyed picking has proven to be very useful in stocks with poor fertilization, if conducted extremely carefully.

Minimize handling and disturbance to only those events that are essential (Jensen and Alderdice, 1983). There are always a great deal of extremely complex biochemical events going on in eggs and, even though the egg is very robust much of the time, a perfectly healthy egg can be killed if the timing of a hard bump occurs at a sensitive period for only one of its millions of cells.

Stimulate development

While the egg is encased in its shell, it might be possible to provide it with some ions or nutrients that will aid in its development. Once the shell is gone, alevins are much more intimately connected with, and sensitive to, the water around it. Treatment with hormones at this stage can alter the sexual development of salmon and it is likely that other chemicals can be utilized by the alevin in its development.

Experiment with adding nutrients, hormones and ions to the incubation water to see if they direct development closer to the incubation target.

Recirculation

Many of the suggested improvements to the environment for adult holding and incubation listed above involve altering the basic characteristics of the water used in the hatchery. While this might be considered to be a shopping list for the type of water that would make up the perfect water supply (but impractical to implement in facilities that do not have the suggested type of water), most of the changes described above can be accomplished fairly easily at any hatchery using water recirculation technology. In fact, complete control of the incubation environment probably depends on the application of water reuse, since to alter the characteristics of any flow-through supply to such an extent would be prohibitively expensive.

The appeal of recirculation for incubation water is that it is much simpler to treat incubation waste water than it is to treat rearing waste water. During rearing, massive amounts of extraneous material is added to the system in the form of feed, and over half of the feed is not incorporated into fish flesh and becomes waste, mainly solids (uneaten food and feces) and ammonia (Timmons and Losordo, 1994). During incubation, no extra material is added to the system whatsoever, and only a very small fraction of the existing egg is excreted as waste, mainly ammonia. For a short period of time, the egg shells are shed and can be removed either as solids, or after they have disintegrated, as a foam fraction. This means that a recirculation plant for an incubation system needs to be much simpler, smaller and cheaper than one for a rearing system.

Conclusion

Fish culturists have the opportunity to make major improvements in the quality of incubation in salmon hatcheries. Any hatchery with a history of poor incubation success should take a more active role in the control of the physical, chemical and biological components that make up its system. Recirculating the incubating water can make such control relatively inexpensive.

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MATERNAL STEROIDS IN BROWN TROUT EGGS INFLUENCE JUVENILE BEHAVIOUR AND PHYSIOLOGY

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Populations of juvenile salmonids are characterised by a large degree of individual variation in several behavioural and physiological traits that influence life history strategies (see Metcalfe et al., 1995). Factors influencing the development of such variation around first feeding are not well understood.

Large quantities of maternal hormones are found in newly fertilised teleost eggs, and may play a role in the endocrine control of early development (Brooks et al., 1997). Our study investigated whether variation in cortisol (F) and testosterone (T) levels in brown trout eggs could result in individual variation in juvenile physiology and behaviour.

Methods

Eggs were stripped from five mature female brown trout and fertilised with milt pooled from three mature males. Each family was divided into three equal-sized groups, and one group from each family immersed for 2 h in a water bath containing 200 μg/l F, 200 μg/l T, or no steroid. All groups were reared in separate baskets in hatchery troughs until first feeding, when they were transferred to hatchery tanks and reared under identical conditions.

Eggs were sampled from each group pre-fertilisation, immediately after the 2-hour immersion period, and two and seven days after treatment. Egg T and F

concentrations (ng/egg; not corrected for recovery efficiency) were measured using radioimmunoassay.

Ten individuals from each group were sampled at hatching, and an estimate of yolk-sac utilisation (ratio of yolk-sac larva:larva weight;YSL:L) was calculated for each individual.

At 3-4 months beyond first feeding, the weight-corrected standard metabolic rates (rSMR) of 20 fish from each treatment group were measured following the methods of Cutts et al. (1998). At the same time, triads comprising size-matched siblings, one from each treatment group, were introduced into sections of an artificial stream, and behaviours, body colour, and position were scored for 24 hours. Dominance ranks within each triad were assigned on the basis of the observation scores. Twenty-four triads were observed within each family.

At 5 months after the onset of exogenous feeding, the remaining fish (n>60/group) were killed, measured and sexed. A condition factor (100 x weight/length^3) was calculated for each individual.

Data were examined for treatment effects using ANOVA, GLM with binary error structure (sex ratios), and polytomous logistic regression (dominance relationships). Correlation was used to examine associations between variables and egg steroid concentrations. A *p*-value less than 0.05 was considered significant.

Results

Hormone levels in eggs before and after treatment are shown in Table 1. Control T levels varied significantly between families. Levels of T and F in control eggs were negatively correlated. Treatment significantly elevated egg steroid levels above controls for at least 7 days post-treatment. Testosterone levels remained constant over this time, while 57-84% of F in F-treated eggs had cleared within 2 days. Steroid uptake varied between families (Table 1), but was not related to egg size, hydration or initial steroid levels. Elevated levels of T were approximately physiological, as were F levels by 2 days post-treatment. Treatment did not affect mortality rates, which were very low in all groups.

Table 1. Egg parameters for each family. Hormone concentrations (ng/egg) are from eggs immediately post-treatment, except where indicated (n=6).

	Family				
	1	2	3	4	5
Control F	0.19	0.17	0.10	0.25	0.12
Treated F	0.63	0.47	0.59	0.82	1.10
Uptake (ng)	0.44	0.30	0.49	0.57	0.98
% Increase	232	176	490	228	817
2 d post-treated F	0.21	0.20	0.11	0.30	0.18
Control T	0.07	0.16	0.12	0.02	0.13
Treated T	0.30	0.34	0.59	0.20	0.21
Uptake (ng)	0.23	0.18	0.47	0.18	0.08
% Increase	329	113	392	900	62

There were significant inter-family differences in control levels of some physiological variables measured. Cortisol or T treatment could affect yolk-sac absorption at hatching, and metabolic rate, size and dominance relationships of juveniles. Treatment had no affect on sex ratios. The effects of treatment were not consistent between families; family-specific treatment effects are shown in Table 2.

Groups with lower mean T concentrations had higher juvenile metabolic rates and relatively less yolk at hatching. Although not significant, higher egg F concentrations appeared to have the same affect on yolk-sac absorption, and may also be associated with higher female:male ratios and juvenile condition factors.

Conclusion

We suggest that naturally occurring variation in levels of maternal F and T in brown trout eggs can lead to variation in offspring physiology and behaviour, but that the effect of hormone concentration is dependent on interaction with other genetic or non-genetic parental contributions to the offspring. The degree of inter-family variation found here has implications for experimental design.

Table 2. Results of within-family comparisons by treatment group. C: control, F: cortisol-treated, T: testosterone-treated, N/S: non-significant.

	Family				
	1	2	3	4	5
YSL weight (mg)	N/S	C>T	N/S	N/S	N/S
L weight (mg)	N/S	N/S	C>T	T>F	C>T
YSL:L	N/S	N/S	T>F,C	F>C>T	T>F,C
rSMR (ml $O_2.h^{-1}$ $x10^{-3}$)	N/S	N/S	N/S	C>F,T	N/S
Final weight (g)	N/S	N/S	T>C	F>C,T	F>C,T
Final length (cm)	N/S	N/S	T>C	F>C	F>C,T
Condition factor	N/S	N/S	T>C	F>T	F>C,T
Sex ratio	N/S	N/S	N/S	N/S	N/S
Dominance	T>C	N/S	N/S	N/S	N/S

Acknowledgements

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WINSIRP: NEW MICROSOFT WINDOWS®-BASED SALMONID INCUBATION AND REARING PROGRAMS, DESIGNED FOR MICROSOFT EXCEL ®.

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Abstract

Predictive models were developed to assist salmonid fish culturists and biologists with a wide range of fish culture problems by McLean et al. (1991). The species modelled were chinook (*Oncorhynchus tshawytscha*), chum (*O. keta*), coho (*O. kisutch*), pink (*O. gorbuscha*), sockeye (*O. nerka*), and steelhead or rainbow trout (*O. mykiss*). These models focussed on incubation, dissolved oxygen during rearing, and excess total gas pressure and they were incorporated into a package of computer programs for PC-compatible computers titled SIRP (i.e. Salmonid Incubation and Rearing Programs) that was easy to use (Jensen et al., 1992). Since that time computer user-interfaces have changed and part of the programs did

not function properly after Y2K. Hence, these programs, now called WinSIRP, have been updated to run in Microsoft Windows® 9X using Microsoft Excel® 97, or later. Additional features in the incubation programs include information on ammonia excretion rates, mechanical shock egg sensitivity, and temperature warnings. This paper discusses the application of the Incubation portion of the WinSIRP programs.

Introduction

Predictive models were developed to assist salmonid fish culturists and biologists with a wide range of fish culture problems by McLean et al. (1991). The species modelled were chinook (*Oncorhynchus tshawytscha*), chum (*O. keta*), coho (*O. kisutch*), pink (*O. gorbuscha*), sockeye (*O. nerka*), and steelhead or rainbow trout (*O. mykiss*). These models focussed on incubation, dissolved oxygen during rearing, and excess total gas pressure. Hence, they allowed predictions of how fish may interact with the culture environment. These models were incorporated into a package of computer programs for PC-compatible computers titled SIRP (i.e. Salmonid Incubation and Rearing Programs) that was easy to use (Jensen et al., 1992). The programs dealt with:

1. the effect of temperature and ambient dissolved oxygen supply on egg and larval development rates of 6 *Oncorhynchus* species,
2. the influence of temperature, fish size and ration level on the rearing capacity of a water supply and
3. gas supersaturation and its effects on fish health.

The programs were originally designed to work as a "stand-alone" package that worked like a typical spreadsheet program. Since that time, computer user-interfaces have changed and part of the programs did not function properly after Y2K. Hence, these programs, now called WinSIRP, have been updated to run in Microsoft Windows® 9X using Microsoft Excel® 97, or later.

In the Incubation Program in SIRP, the models would calculate development time to key embryonic stages, oxygen consumption (Ro, mg/1000/hr), critical oxygen values (Pc, the dissolved oxygen level below which dependent respiration occurs), and predicted oxygen concentration in the incubator effluent. Additional features in the new WinSIRP Incubation Programs include information on ammonia excretion rates, mechanical shock egg sensitivity, and temperature warnings. Also, a series generic treatment scenarios for circular

and raceway ponds have been included that allow fish culturists to quickly calculate chemical treatment concentrations and flow rates for numerous combinations of pond size, flow rates, and fish density. This paper discusses the application of the Incubation portion of the WinSIRP programs.

Additional Features in the Incubation Portion of WinSIRP

1. Ammonia excretion rates

Mclean and Lim (1985) measured ammonia excretion in chinook eggs and alevins in a hatchery production setting. An empirical ammonia excretion model was developed from their data for a given egg size and temperature. The first relationship determined is shown in the following equation

$$Y = \text{-}0.002805 + 0.0013037\text{X} + 5.916\text{E} - 06\text{X}^2 \qquad \text{(Eq. 1)}$$

where Y is NH_3-N (μg/g wet wt/hr), X is ATUs (°C-days), and R^2=0.9576. This relationship was then used to generate weekly predictions of NH_3-N which we modelled against oxygen consumption (Ro, mg O_2/1000 eggs or alevins/hr); from the SIRP predictions) for chinook to yield the following equation

$$Y = 0.072836 + 111.638759/(1 + (\text{X}/11328.08987)^{-0.647114}) \qquad \text{(Eq. 2)}$$

where Y is NH_3-N (μg/g wet wt/hr), X is Ro (mg O_2/1000 eggs or alevins/hr), and R^2=0.99932.

Since there currently are no similar data for the other salmonid species included in SIRP, we have used the ammonia-Ro relationship developed for chinook and made the assumption that, at corresponding stages of development, the other 5 species will exhibit similar metabolism to chinook. Therefore, equation 2 is used to predict ammonia excretion for the other 5 salmonid species based on their calculated Ro values.

2. Mechanical Shock Sensitivity of eggs

Mechanical shock refers to the force on eggs that occurs as a result of disturbance to eggs. Disturbances can occur during handling (i.e., egg removal from female, pouring eggs into incubators, egg transportation, egg picking) or from outside sources such as pile driving or blasting and seismic shock. Jensen

and Alderdice (1983, 1989) reported changes in shock sensitivity in units of energy (ergs) transferred to eggs on impact, based on the drop height that caused 50 % and 10 % mortality. Their work was conducted at 10°C. Assuming that the changes in mechanical shock sensitivity are associated mainly with stage of development, then it follows that the data of Jensen and Alderdice (1983, 1989) can be reported in terms of ATUs. Hence, the LC50s and LC10s (i.e. drop heights causing 50% and 10% mortality) were modelled against ATUs and have been included in WinSIRP as warnings during the sensitive periods of egg development during incubation. In the incubation program that predicts weekly changes in embryonic development rate and metabolism, an additional column has been added that warns of mechanical shock sensitivity based on LC50s. The warnings are as follows:-

1. If the LC50 is greater than 115 cm then the warning is " **Shock resistant**"
2. If the LC50 is between 115 and 50 cm then the warning is "**Sensitive**"
3. If the LC50 is between 50 and 10 cm then the warning is "**Very Sensitive**"
4. If the LC50 is less than 10 cm then the warning is "**Extremely Sensitive**"

Figure 1 illustrates the changes in egg sensitivity in chinook salmon eggs.

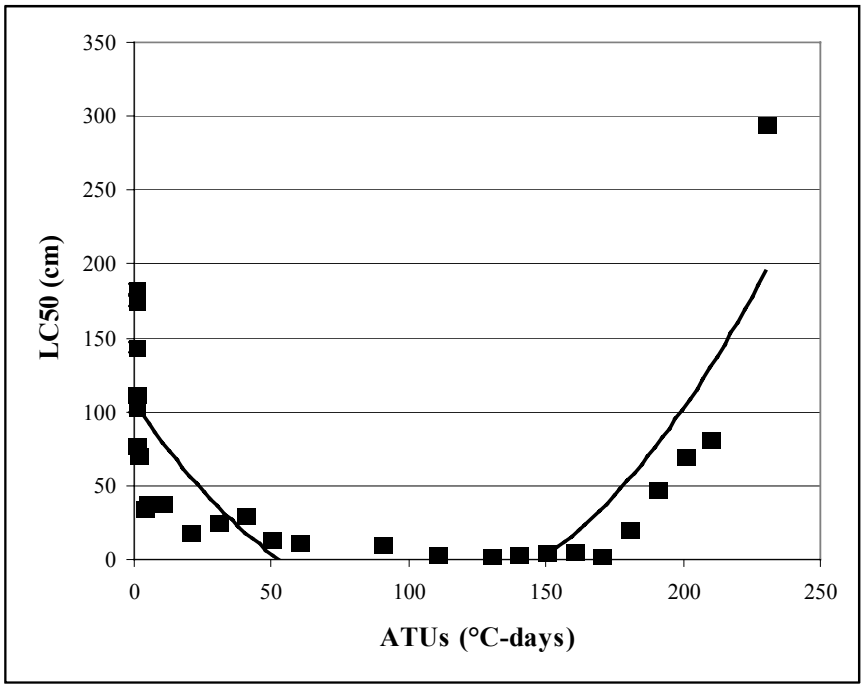

Figure 1. Chinook LC50 (i.e. drop height causing 50% egg mortality, cm) in relation to development time (ATUs). The solid line represents the equation $y=105.02387-2.70227x+0.01344x^2$; $R^2=0.6546$.

In addition, new units of egg sensitivity, called LC10 Velocity (i.e. the final velocity, cm/sec, reached when eggs are dropped causing 10% mortality), have also been developed to help in determining the potential hazards of seismic shock (see the Extended Abstract "NEW MECHANICAL SHOCK SENSITIVITY UNITS IN SUPPORT OF CRITERIA FOR PROTECTION OF SALMONID EGGS FROM BLASTING OR SEISMIC DISTURBANCE." by Jensen included elsewhere in this symposium). Therefore, for those that require egg sensitivity in relation to seismic disturbance an additional worksheet has been included in WinSIRP that yields expected egg mortality in response to pressure wave velocity.

3. Egg Mortality at High and Low Temperatures

Many researchers have studied and reported on pacific salmon egg mortality at high and low temperatures. Egg mortality data for the 6 *Oncorhynchus* species from Beacham and Murray (1985, 1986, 1988, and 1989), Murray et al. (1990), and Velsen (1987) were consolidated for each species and modelled using a 2nd order polynomial since the data exhibited a typical parabolic shape, with increased mortality at high and low temperature extremes. Chinook egg mortality response data and the parabolic model are shown in Fig. 2 to illustrate this. The model parameters for all 6 species are tabulated in Table 1.

Table 1 . Parabola (i.e. $y=a+bx+c^2$) model parameters for the 6 salmonid species.

Parameter	Chinook	Chum	Coho	Pink	Sockeye	Steelhead or Rainbow
a	117.2522	55.920727	31.467233	114.91779	41.751667	56.168418
b	-25.35362	-11.09208	-12.20988	-25.06966	-8.556693	-13.31631
c	1.3003801	0.5849235	1.0584796	1.3147656	0.542333	0.8348565
R^2	0.7498169	0.3967366	0.8048845	0.6631796	0.2434187	0.5075305
n	101	58	96	66	63	16

There are a number of observations to be made from Table 1. Differences in R^2 values likely are due to variations in quantity of data, with the number of data records (n) for each species varying from 16 to 101. Also, data were compiled from many different sources. Therefore, we may be seeing stock differences as well as differences in how constant the temperatures were during egg incubation. In addition, there were differences in the distribution of temperatures to which the different species were exposed. Finally, these data represent the total mortality for eggs from fertilization to hatch in response to exposure to constant temperatures. Hence, since there are many variables that have influenced the predictive power of these models, it was decided that broad temperature warnings (see the vertical arrows in Fig. 2) should be given based on the models in Table 1. Four levels of temperature warnings were chosen, namely: -

1. If, at a given incubation temperature, the parabola model predicts a value of 20% mortality or less, then the following warning is given “Expect 20% or less egg mortality at this temperature”.

2. If, at a given incubation temperature, the parabola model predicts a value between 20 and 30% mortality, then the following warning is given "Expect 20 to 30% egg mortality at this temperature".
3. If, at a given incubation temperature, the parabola model predicts a value between 30 and 50% mortality, then the following warning is given "Expect 30 to 50% egg mortality at this temperature".
4. If, at a given incubation temperature, the parabola model predicts a value greater than 50% mortality, then the following warning is given "Expect greater than 50% egg mortality at this temperature".

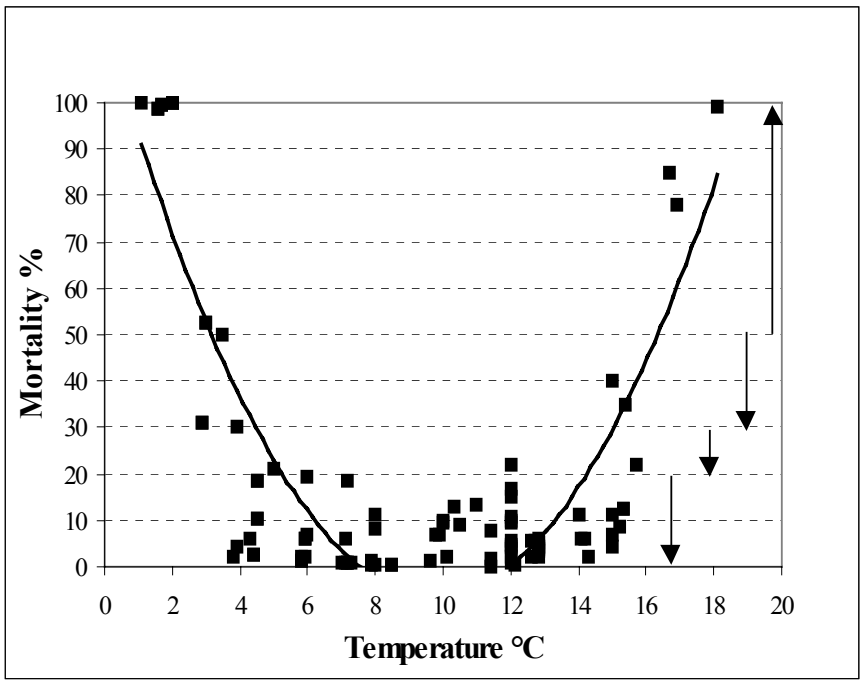

Figure 2. Chinook egg mortality in response to constant temperature from fertilization to hatch. The solid line represents the equation $y=117.252220-25.3536221x+1.30038x^2$; $R^2=0.7498$. The vertical arrows represent warning levels described in the text above.

Description of the Incubation Portion of WinSIRP

In the remainder of this paper, a brief description of the Incubation portion of WinSIRP follows.

System Requirements

WinSIRP has been installed and tested to run successfully on a number of PCs with Windows 95 and Excel 97. It should be compatible with all newer versions of Windows and Excel. The minimum CPU type is a Pentium or equivalent with a minimum speed of 133 MHz and a minimum of 32 Megs of RAM. The program installation set up files can be obtained on a CD by contacting the principle author. Also, look for a downloadable version of this program in the "What's New" section of the Pacific Region's Fisheries and Oceans Canada Aquaculture web site
(i.e. http://www-sci.pac.dfo-mpo.gc.ca/aqua/english/default.htm).

Starting WinSIRP and Menu Buttons

Once WinSIRP has been installed, the user starts WinSIRP from the Programs list. After noting the information on the introduction screen and clicking OK the user will see a typical Excel screen, with a brief introductory description of the programs. To use the various components of WinSIRP, new menu buttons have been added to the toolbar menu. These menu buttons are SIRP, SIRP Plots, and SIRP HELP. Selecting SIRP reveals a menu of choices shown in Fig. 3 below.

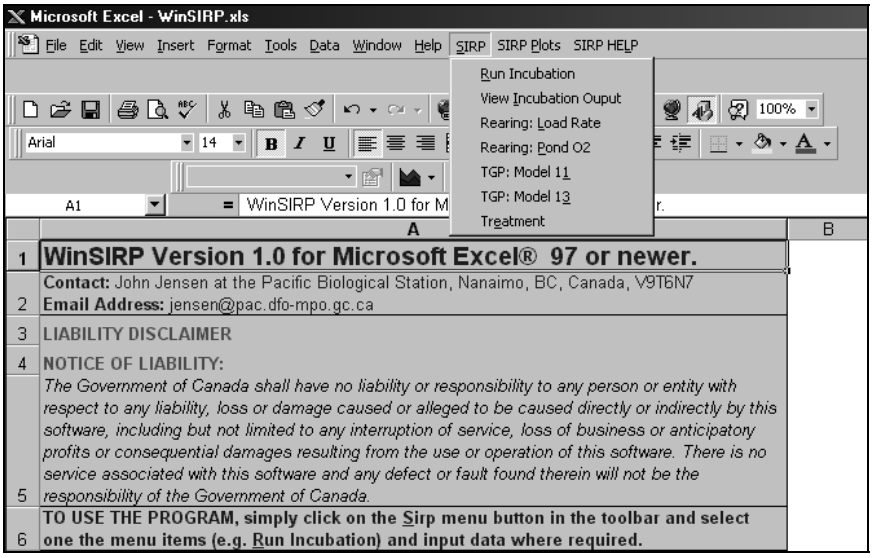

Figure 3. First worksheet when WinSIRP is started illustrating the drop-down menu for SIRP, one of the 3 new menu buttons.

Incubation Programs

To start the Incubation programs, the user selects Run Incubation. This opens the input menu screen…

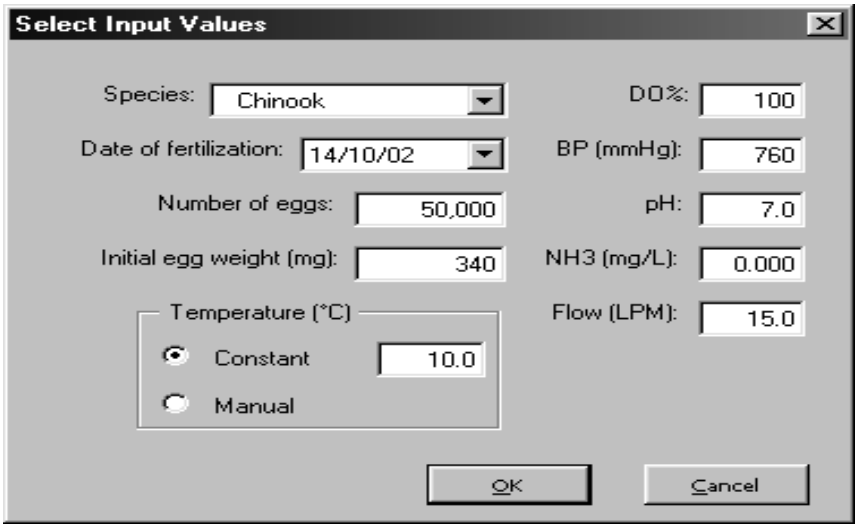

Figure 4. Input menu box with values used for the chinook egg incubation example below.

The user first chooses a species from a drop-down list of the 6 salmonid species (i.e. chinook, chum, coho, pink, sockeye, and steelhead). The fertilization date then is selected by using the calendar presented.

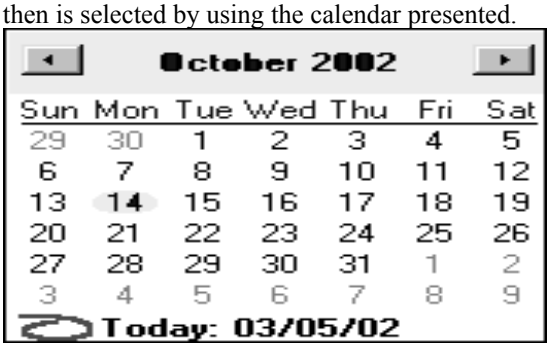

Figure 5. Calendar for selecting fertilization date.

The next choice to make is egg size, which is important for calculations of development and ammonia production. The user can then either select "constant" and type in a constant temperature or can select "manual" and input a series of average weekly temperatures. The user can also change important inflow water quality parameters or "inputs" in the menu. These "inputs" are

dissolved oxygen (DO), barometric pressure (BP), pH, ammonia (NH_3-N), and water flow rate. When all inputs have been selected the user clicks OK and a series of calculations are performed, based on the models described by McLean et al. (1991) and Jensen et al.(1992). Values for developmental stages (i.e. beginning of epiboly, yolk plug closure, eyed, hatch, and maximum alevin wet weight "MAWW"), oxygen consumption (Ro), critical oxygen level (Pc), (DO) at inflow and outflow, ammonia production, mechanical shock sensitivity of eggs, and temperature warnings for eggs are then displayed for weekly time periods in the "Incubation Output" worksheet. To illustrate the many potential uses for this worksheet the following example with chinook is presented.

Chinook Egg Incubation Example

Using the input values in Figure 4, resulted in the following worksheet.

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Output Table							Species	Chinook						
Stage	**ATU**	**Days**	**Mean Temp**	**Date**	**Stage Description**		Fertilization date	14-Oct-02						
0		0		14-Oct-02	Fertilization		Number of eggs	50000						
1	55	5.5	10.0	19-Oct-02	Begin Epiboly		Egg weight	340						
2	135	13.5	10.0	27-Oct-02	Yolk Plug Closed		DO%	100						
3	252	25.2	10.0	08-Nov-02	Eyed		BP	760						
4	526	52.6	10.0	05-Dec-02	50% Hatch		pH	7.0						
5	964	96.4	10.0	18-Jan-03	MAWW		NH3	0.0						
							Flow	15.0						
Species: Chinook							Temperature	10.0						
Weekly Mean Temp. (°C)	**Mean Flow Rate (LPM)**	**pH**	**Running Mean Temp. (°C)**	**Date**	**Days from fertilization**	**ATUs**	**Stage**	**Ro mg/1000 eggs hr**	**Pc (mg/L)**	**DO *IN* (mg/L)**	**DO *OUT* (mg/L)**	**NH3-N ug/g wet wt/hr**	**Total NH3-N *OUT* (mg/L)**	**un-ionized NH3-N *OUT* (ug/L)**
10	15	7.00	10.0	14-Oct-02	0	0.0								
10	15	7.00	10.0	21-Oct-02	7	70.0	Begin Epiboly	0.55	2.00	11.26	11.23	0.25	0.01	0.01
10	15	7.00	10.0	28-Oct-02	14	140.0	Yolk Plug Closed	0.62	2.42	11.26	11.22	0.27	0.01	0.01
10	15	7.00	10.0	04-Nov-02	21	210.0		2.00	3.59	11.26	11.14	0.49	0.01	0.02
10	15	7.00	10.0	11-Nov-02	28	280.0	Eyed	4.56	4.76	11.26	11.00	0.78	0.02	0.03
10	15	7.00	10.0	18-Nov-02	35	350.0		8.67	5.92	11.26	10.77	1.14	0.03	0.05
10	15	7.00	10.0	25-Nov-02	42	420.0		14.63	7.07	11.26	10.44	1.56	0.03	0.06
10	15	7.00	10.0	02-Dec-02	49	490.0		22.79	8.22	11.26	9.99	2.05	0.04	0.08
10	15	7.00	10.0	09-Dec-02	56	560.0	50% Hatch	33.44	4.40	11.26	9.40	2.59	0.06	0.11
10	15	7.00	10.0	16-Dec-02	63	630.0		46.91	4.40	11.26	8.65	3.19	0.07	0.14
10	15	7.00	10.0	23-Dec-02	70	700.0		63.49	4.40	11.26	7.73	3.84	0.09	0.17
10	15	7.00	10.0	30-Dec-02	77	770.0		83.49	4.40	11.26	6.62	4.54	0.12	0.22
10	15	7.00	10.0	06-Jan-03	84	840.0		107.20	4.40	11.26	5.30	5.29	0.15	0.28
10	15	7.00	10.0	13-Jan-03	91	910.0		134.91	4.40	11.26	3.76	6.08	0.18	0.34
10	15	7.00	10.0	20-Jan-03	98	980.0	MAWW	166.92	4.40	11.26	1.98	6.91	0.22	0.41

Figure 6. Incubation Output worksheet illustrating Ro, Pc, Do in, DO out, and NH_3 production for chinook eggs at 10°C.

To illustrate the cause and effect relationships of the inputs (i.e. temperature, flow, DO, pH, and the number of eggs) on the outputs (i.e. Ro, Pc, DO out, and NH_3 production) four figures are included. The "Incubation: Ammonia Plot"

(Fig. 7) illustrates the increase in ammonia production from fertilization to MAWW.

The user can then change inputs such as number and size of eggs, flow rate, initial NH_3 level, and pH and immediately see the consequences on ammonia production. For example, if the source water contains a background level of 1 mg/L and a pH of 7.9 the resultant ammonia in the outflow of the incubator (i.e. un-ionized NH_3-N) increased from a maximum of 0.41 μg/l (Fig. 7) at MAWW to 17.75 μg/l (Fig. 8).

Similarly, oxygen consumption changes from fertilization to MAWW are illustrated in Fig. 9. Notice, in Fig. 9, that the DO OUT (mg/L) curve drops below the Pc (mg/L) level at 91 days post-fertilization. To fix this serious risk to late yolk sac alevins, we could increase the flow, decrease the temperature, add supplemental oxygen, or reduce the number of alevins in the incubator. For this example the flow was increased from 15 LPM to 25 LPM. The resultant improvement in DO OUT is shown in Figure 10.

This example was meant to illustrate how quickly and easily one can view the impact of the important factors that influence salmon egg and larval development and metabolism. Furthermore, the worksheets have been designed to calculate critical values of oxygen consumption and ammonia production in ways that are useful for fish culturists, field biologist, and researchers. By presenting the numerous models in an up-to-date Windows® Microsoft Excel® format, the dynamic nature of the input and output factors can easily be explored.

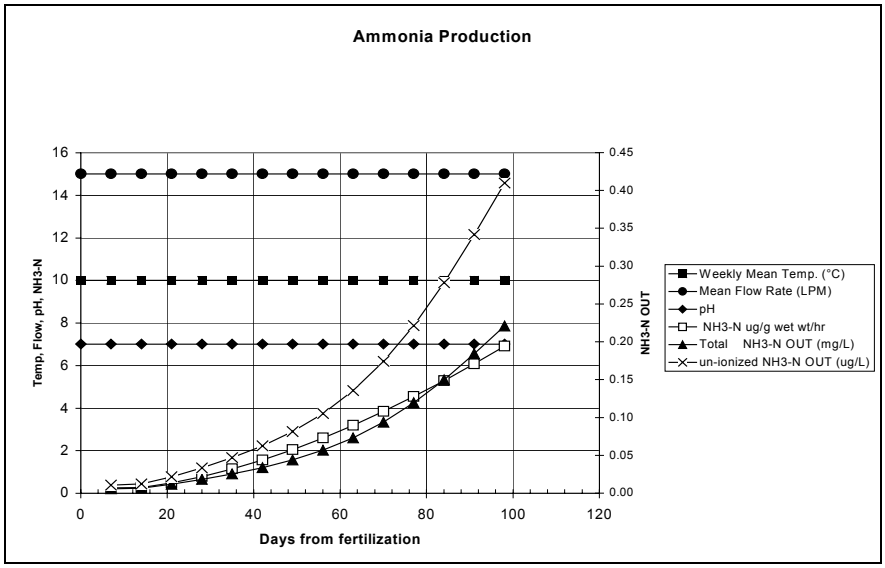

Figure 7. Incubation: Ammonia Plot. Ammonia production from fertilization to MAWW.

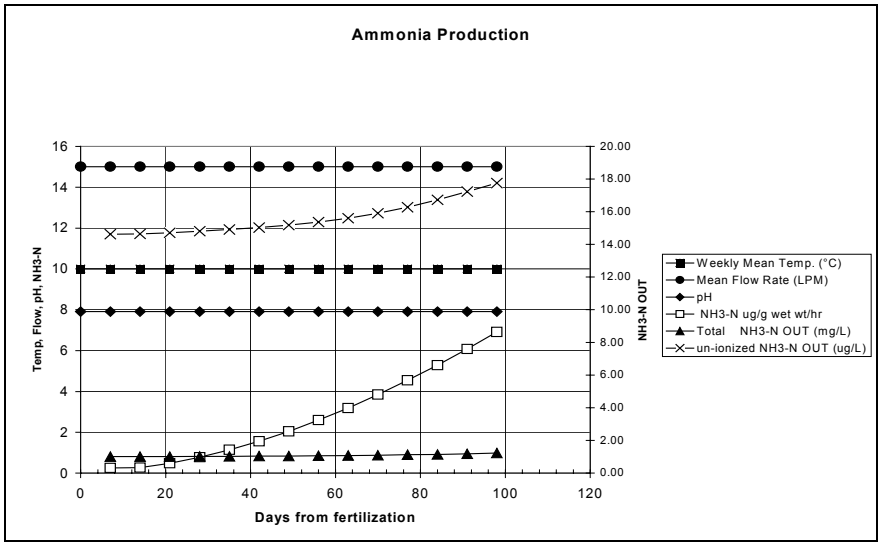

Figure 8. Incubation: Ammonia Plot with initial background NH_3-N level changed to 1 mg/L and pH changed to 7.9.

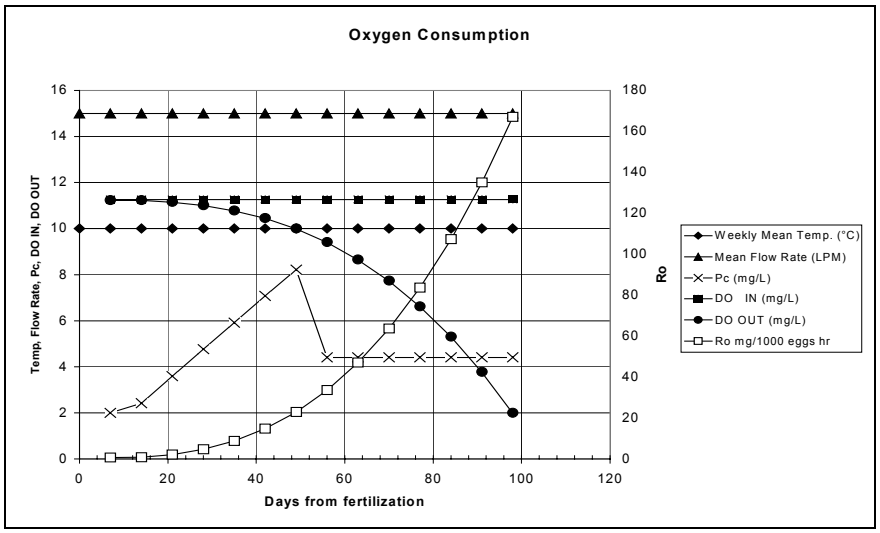

Figure 9. Incubation: Oxygen Plot showing Ro (mg O_2 consumed per 1000 eggs per hr) in response to temperature and flow.

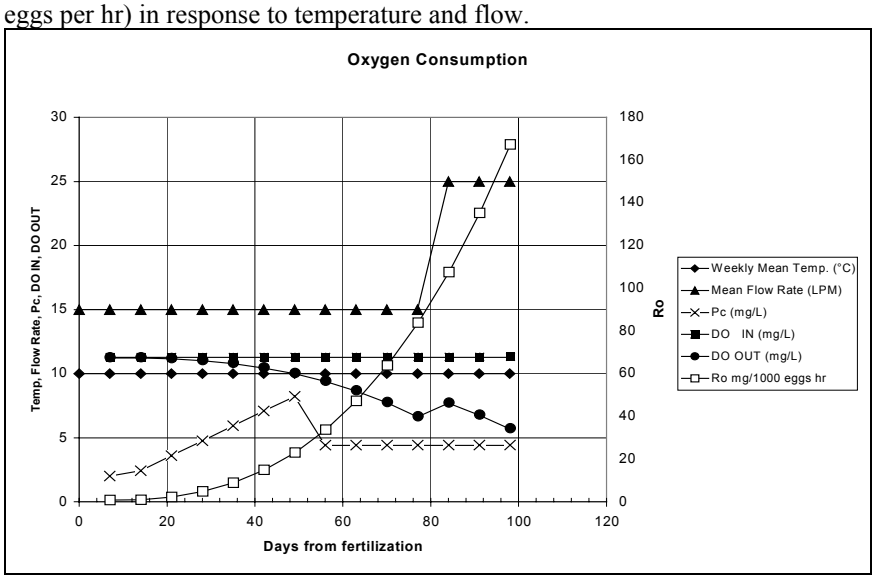

Figure 10. Incubation: Oxygen Plot illustrating the improvement in the DO OUT levels by increasing flow from 15 to 25 LPM in the last 3 weeks.

Acknowledgements

We gratefully acknowledge Bill Damon and Georgia Borgford for their helpful comments and for testing initial trial-versions of these programs.

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THE EFFECT OF TEMPERATURE AND YOLK TRIIODOTHYRONINE ON THYROID HORMONE RECEPTOR EXPRESSION IN RAINBOW TROUT EMBRYOS

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EXTENDED ABSTRACT - DO NOT CITE

Introduction

The influence of maternal thyroid hormones on the development of teleost fish embryos is still unresolved. Studies involving the immersion of hatched fish embryos in solutions of thyroid hormones have yielded conflicting results, but have shown that thyroid hormones can influence development (see Leatherland, 1994, for review). This suggests that thyroid hormone receptors are present at this early stage, but this method of exposure to thyroid hormone is not biologically meaningful, as the thyroid hormone enters the blood of the embryos directly through the gills, and can not be regulated. Other studies that have attempted to elevate yolk thyroid hormone levels through elevation of broodstock plasma levels, provide a more realistic method of embryo exposure (see Leatherland, 1994, for review). However, this method of egg yolk elevation has not been successful with salmonid species to date. We have developed a new method of elevating egg yolk thyroid hormone levels, in order to provide a more biologically-relevant method of exposing the embryos to elevated yolk thyroid hormone levels.

We were particularly interested in the influences of yolk triiodothyronine (T_3) on

trout embryo whole body thyroid hormone levels and on the expression of thyroid hormone receptor (TR) mRNA. We anticipated that the TR mRNA levels would prove to be a more sensitive marker for studying the effects of yolk thyroid hormones on embryo development. Two types of TR have been found, TRα and TRβ. Both are differentially expressed in flounder, but relatively little is known of TR expression in salmonid species (Yamano and Miwa, 1998).

Environmental temperature is a major factor regulating the development of poikilothermic teleost fish. Positive correlations have been found between temperature and developmental rates for many species (Blaxter, 1988). If yolk T_3 influences thyroid hormone clearance or TR gene expression, then we were curious to see if temperature would have a predictable effect on this relationship.

This study explores the relationship between yolk T_3, whole body thyroid hormone levels, and thyroid hormone receptor expression in rainbow trout embryos reared at two ambient temperatures.

Materials and Methods

Two separate, but similar, experiments were carried out. One study examined the clearance of yolk thyroid hormones while the other study looked at changes in thyroid hormone receptor expression . For both experiments, eggs from 5 or 6 female broodstock were pooled, divided into six groups and treated with 0, 30 or 150 ng/ml T_3 in ovarian fluid for 3 hours. The eggs were then fertilized with milt pooled from 5 male broodstock. Three groups of eggs treated with 0 (control), 30 (T_3 low)or 150 (T_3 high) ng/ml T_3 were raised at 8.5°C, and the other 3 identical groups were raised at 5.5°C. Samples were taken from each of the 6 groups after fertilization for initial egg yolk T_3 levels.

Samples for thyroid hormone concentrations were taken at 5 different stages of development, based on results found in Raine and Leatherland (1999); 1) pre-phase 1 (before endogenous thyroid hormone synthesis), 2) phase 1 (endogenous thyroid hormone synthesis occuring), 3) phase 2 (endogenous thyroid hormone release occuring), 4) swim-up, and 5) complete yolk absorption.

Samples to determine mRNA levels of TRa and TRB were taken just prior to the onset of endogenous thyroid hormone production, as determined in Raine and Leatherland (1999). Embryos were dissected free of the yolk and chorion and snap -frozen in liquid N_2. RNA was then extracted from the embryos, cDNA generated, and real-time PCR performed, using primers designed for TRα and TRβ.

Results and Discussion

Yolk T_3 levels were elevated in T_3 treated eggs as expected, but the increased concentration of yolk T_3 quickly disappeared over about 14 days, in the embryos raised at both ambient temperatures (Figure 1). The resulting thyroid hormone

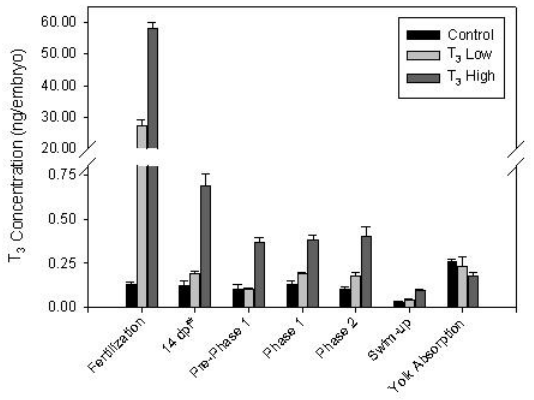

Figure 1. Whole body T_3 levels from rainbow trout embryos with 3 different amounts of yolk T_3, at several stages of development, and raised at 5.5°C. Embryos reared at 8.5°C had similar T_3 levels and exhibited the same trend.

levels were similar in eggs treated with different amounts of T_3, although the T_3 content of the eggs with elevated yolk T_3 remained slightly higher, until after endogenous thyroid hormone release (phase 2) was presumed to occur. Thyroxine (T_4) levels increased in embryos after phase 2, presumably reflecting the increase in endogenous thyroid hormone production in the embryos (Figure 2). When

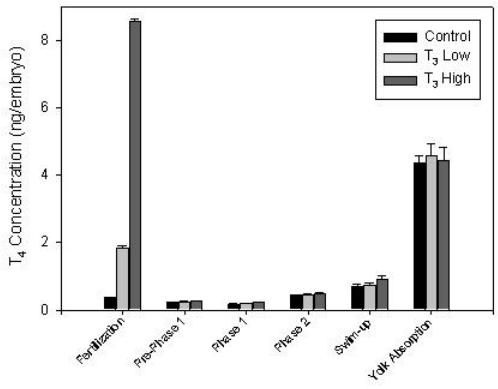

Figure 2. Whole body T_4 levels from rainbow trout embryos with 3 different amounts of yolk T_3, at several stages of development, and raised at 8.5°C. The embryos raised at 5.5°C showed similar T_4 levels and exhibited the same trend.

thyroid hormone levels were compared by developmental stage, the thyroid hormone profiles of the embryos reared at 5.5°C and 8.5°C matched up, showing that temperature has a predictable affect on the whole body thyroid hormone levels.

Egg thyroid hormone levels for this experiment showed the same relationship between treatment levels as found above. The mRNA levels for both TRα and TRβ showed a similar trend between treatments and incubation temperatures. However, temperature appears to alter the effect of the increased yolk T_3 level on TR expression.

Acknowledgements

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NEW MECHANICAL SHOCK SENSITIVITY UNITS IN SUPPORT OF CRITERIA FOR PROTECTION OF SALMONID EGGS FROM BLASTING OR SEISMIC DISTURBANCE

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Mechanical shock refers to the force on eggs that occurs as a result of disturbance to eggs. Disturbances can occur during handling (i.e., egg removal from female, pouring eggs into incubators, egg transportation, egg picking) or from outside sources such as pile driving or blasting and seismic shock. To overcome many of the difficulties and uncertainties of interpreting the egg survival responses to mechanical shock, a device was developed at the Pacific Biological Station in the early 1980s to expose salmonid eggs to standardized, quantifiable shock intensities (Jensen and Alderdice, 1983, 1989). The species tested were chinook (Oncorhynchus tshawytscha), chum (O. keta), coho (O. kisutch), pink (O. gorbuscha), sockeye (O. nerka), and rainbow or steelhead trout (O. mykiss).

Jensen and Alderdice (1983, 1989) reported changes in shock sensitivity in units of energy (ergs) transferred to eggs on impact, based on the drop height that caused 50 % and 10 % mortality. This standard unit was useful in demonstrating the changes of egg sensitivity during incubation. However, since these papers were published, requests have been received by Jensen to make recommendations about the potential hazards of disturbances such as pile driving or blasting for such activities as road construction and most recently explosive blasting for densification of the earth fill component of a dam.

This abstract describes a new approach to convert the original data, reported by Jensen and Alderdice (1983, 1989), from LC10s (i.e. drop height, cm, causing

10% mortality) to the final velocity (cm/sec) that the eggs reach when dropped from a height resulting in 10% mortality. This new unit of egg sensitivity can then be compared to the peak particle velocity (PPV) criteria of 1.3 cm/sec recommended by Wright and Hopky (1998) for blasting.

Jensen and Alderdice (1983, 1989) used a device developed to expose small groups of eggs to a series of standardized quantifiable shock intensities. The apparatus (Fig. 1) consists of a metal carrier with a slot to hold a petri dish (60-mm diameter x 15 mm) containing a single layer of eggs. The carrier is attached to a release platform by a release trigger. The platform can be moved to any drop height ranging from 0 to 100 cm. The carrier falls freely, guided by two guide wires when the trigger is released. Oversized Teflon sleeves mounted in the carrier guides minimize friction. The egg carrier was designed to come to an abrupt stop upon impact when dropped. This was accomplished by partially filling a hollowed-out portion of the carrier with lead shot. In addition, a 2-mm thick plate of synthetic elastomer, with high impact strength and ability to absorb shock, was fastened to the base to prevent the carrier from bouncing.

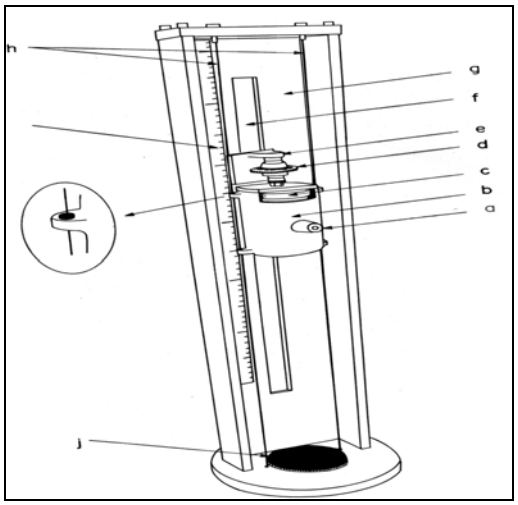

Figure 1. Mechanical shock device for salmonid eggs (from Jensen and Alderdice, 1983). a: handle for raising carrier; b: metal carrier; c: slot for petri dish in position; d: release trigger; e: release platform; f: slot for adjustment of release platform height; g: stage frame; h: metal guide wires; i: 100-cm scale; j: base plate. Inset: showing guide wire passing through Teflon sleeve.

Each shock test consisted of three drop heights (5 – 100 cm) and one control (0 cm); these tests were replicated three times at each time interval. Samples of 20 – 30 eggs were placed in a petri dish, free of surrounding fluid, and then placed in the carrier. The tests were carried out on eggs beginning with un-activated eggs, continued at very short time intervals (i.e. minutes and hours) post fertilization, followed by daily tests until egg sensitivity was no longer measurable.

The advantage of the shock device described herein is that it employs basic principles of physics which allow for the determination and reporting of the results using standard units of measure such as the acceleration (cm/sec2) and velocity (cm/sec) of eggs dropped from various heights, assuming minimal influence of friction. The drop heights that were determined to cause 10% mortality (Jensen and Alderdice, 1989) were used to determine the corresponding final velocity reached by the eggs. The relationship of the parameters of drop height (s; cm), initial velocity (vo; cm/sec), final velocity at time of impact (vt; cm/sec), and acceleration due to gravity (g; cm/sec2) is illustrated in the following equation:

$$vt = (vo^2 + 2 \bullet g \bullet s)^{1/2}$$

where vo=0, g=980. The LC10 velocities were calculated by substituting LC10 drop heights for s.

In this paper the LC10 velocities were modelled in relation to accumulated temperature units (ATUs) instead of days from fertilization. Hence, the resultant equations can be applied to temperatures other than the 10 °C test temperatures, making the models much more versatile at various temperatures (Table 1).

Table 1. Log-linear and parabola model coefficients for LC10 velocities (cm/sec).

Species & Model type	ATUs (°-days)	Model coefficients a	b	c	r^2
Chinook					
Log-linear	0 - 50	191.813486	-27.604286		0.826208
Parabola	50 -230	372.914357	-6.468933	0.029199	0.904477
Chum					
Log-linear	0 - 40	161.876465	-26.359391		0.554601
Parabola	60 -240	203.083120	-3.237649	0.016230	0.925421
Coho					
Log-linear	0 - 50	126.211035	-15.956642		0.649024
Parabola	50 - 180	216.596882	-4.087539	0.021588	0.925421
Pink					
Log-linear	0 - 30	168.386172	-25.844475		0.804388
Parabola	30 - 190	248.262505	-4.233782	0.024102	0.900141
Sockeye					
Log-linear	0 - 50	225.228193	-33.570647		0.729549
Parabola	50 - 200	273.584965	-4.189878	0.023123	0.953769
Steelhead					
Log-linear	0 - 40	138.671408	-22.213301		0.611474
Parabola	40 - 150	284.510542	-6.420712	0.041003	0.909880

[1] Log-linear model: $y = a + b\ln x$

[2] Parabola model: $y = a + bx + cx^2$

To illustrate these changes in egg sensitivity as embryonic development progresses, the predicted LC10 velocities (based on log-linear models and parabolic models as described above) for chinook salmon eggs, are plotted against ATUs (°C-days) from fertilization (Figure 2).

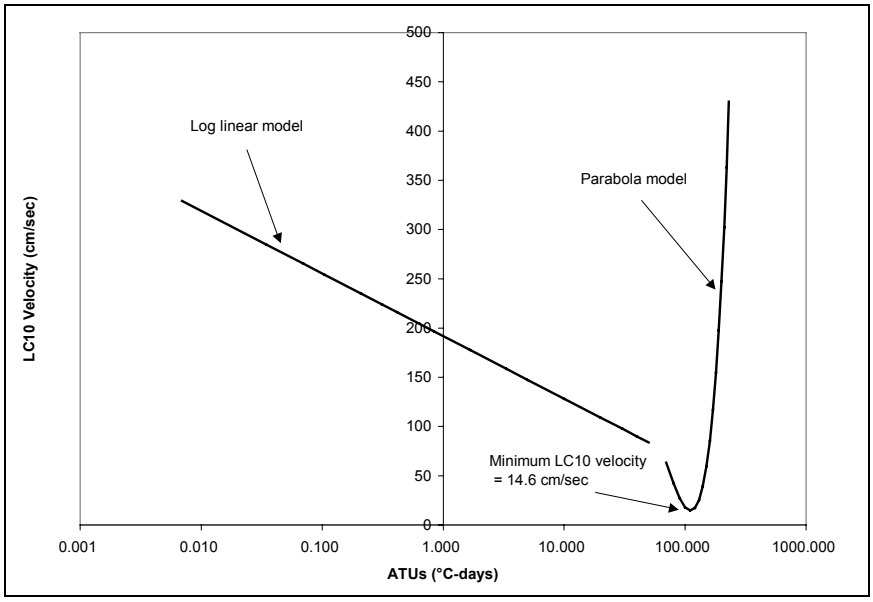

Figure 2. Predicted LC10 velocity for chinook salmon eggs (from model coefficients in Table 1) are plotted against ATUs (°C-days) from fertilization. The minimum LC10 velocity was 14.6 cm/sec. and occurred at 110.8 ATUs.

Wright and Hopky (1998) describe guidelines for protection of fish in response to explosives. In their report they recommend that no explosives should produce a peak particle velocity (PPV) greater than 1.3 cm/sec. Hence, it follows that the LC10 velocities reported herein should be much greater than 1.3 cm/sec. to ensure that no egg mortality occurs. The worst case scenario occurs at the minimum LC10 velocity (i.e. the lowest velocity causing 10 % mortality). The LC10 velocity minima were determined from parabolic models for each species.

These LC10 velocity minima and the ATUs when they occur, for the 6 salmonid species tested, are listed in Table 2.

Table 2. Predicted minimum LC10 velocities (cm/sec) at ATUs post-fertilization. Based on the parabolic equation coefficients from Table 1.

Species	Minimum LC10 Velocity (cm/sec)	ATUs (°C-days)
Chinook	14.6	110.8
Chum	41.6	99.8
Coho	23.1	94.7
Pink	62.3	87.8
Sockeye	83.8	90.6
Steelhead	33.2	78.3

Notice that these values are at least ten times greater than the PPV of 1.3 cm/sec recommended as a safe criterion for the use of explosives by Wright and Hopky (1998). For example, in order to cause 10 % egg mortality in chinook (the most sensitive salmonid species tested) at 111 ATUs (the most sensitive time of development for chinook), the recommended safe criterion of 1.3 cm/sec PPV would have to be exceeded by more than ten times, which it is (i.e. minimum LC10 Velocity is 14.6 cm/sec.). Hence, these new egg sensitivity units are in good agreement with the current guidelines for the use of explosives or other disturbances near salmonid spawning redds.

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DIGITAL PHOTO-MICROSCOPY

OF SABLEFISH (*ANOPLOPOMA FIMBRIA*)

EMBRYONIC DEVELOPMENT

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Abstract

Sablefish (*Anoplopoma fimbria*) eggs were incubated at temperatures ranging from 4 to 8 °C. Digital photo-microscopy was employed to obtain an accurate determination of embryonic development rates of surviving embryos and larvae at the various temperatures. Representative pictures of key developmental stages are presented.

Introduction

Research on sablefish (*Anoplopoma fimbria*) eggs and larvae has been ongoing at the Pacific Biological Station, Nanaimo, BC, Canada, since the late 1980s (Alderdice et al., 1988a, 1988b; Bowden et al., 1990; Jensen and Groot, 1990; Jensen et al., 1992; Whyte et al., 1994; Clarke and Jensen, 2001). In their paper on preliminary trials on incubation of sablefish eggs, Alderdice et al. (1988a) documented embryonic development prior to hatch at several temperatures (i.e. 3.6 to 7.9 °C) and included photo-microscopy images for some stages. We incubated sablefish eggs at similar temperatures (i.e. 4 to 8 °C) to obtain more information on early embryonic development rates in relation to temperature. In addition, we present new digital photo-microscopy images of both pre- and post-hatch embryonic development.

Materials and Methods

Small batches of fertilised sablefish eggs were obtained from broodstock held at the Pacific Biological Station and placed in 4-litre beakers filled with 34 ppt filtered (0.2 μ) seawater. The beakers were held in temperature-controlled (± 0.1 °C) water baths (i.e. 4, 5, 6, 7, and 8 °C). We attempted to replace the water periodically to prevent fouling in the static water. This was not completely successful and eggs only survived to day 4 in 8 °C, and to day 5 in 7 °C. For the 6 °C treatment, eggs and larvae were collected periodically every few days from a flow-through incubator to ensure that samples were not adversely influenced by poor water quality. In the lower temperatures eggs survived longer since bacterial growth was slower. Hence eggs survived in the static water temperatures of 6,5, and 4 °C for 7 days post-fertilization. Developing eggs were sampled at various intervals, initially at 4-hr intervals, from each temperature, and were photographed using a Nikon Coolpix 990, 3.1 megapixel, digital camera mounted on a Nikon SMZ1000 stereo microscope equipped with a diascopic stand that utilises Nikon's Oblique Coherent Contrast illumination system. This system was ideal for photographing the transparent eggs and developing embryos.

Results

Pictures of embryonic development were obtained from early cell division to almost complete yolk absorption in hatched larvae. A series of pictures of developmental stages of eggs and larvae from fertilization to near yolk absorption (38 days post fertilization or 228 °C-days at 6°C) have been compiled from the hundreds of digital pictures obtained, and some of the stages are illustrated in Figs 1 to 8.

To aid researchers and fish culturists, the development time for the above stages have been mathematically modelled, based on the 5.3 °C trial from Alderdice et al. (1988a) for eggs and from Clarke and Jensen (2001) for larval yolk absorption, to allow quick and accurate predictions of when these stages will occur at temperatures ranging from 4 to 8 °C. These predictions are tabulated as development time in hours and accumulated temperature units (ATUs; °C-days) in Table 1.

Figure 1. Sablefish egg at the 2-cell stage (i.e. first cell division has occurred). This occurs at 1.3 °C-days.

Figure 2. Sablefish egg at the 8-cell stage. This occurs at 2.9 °C-days.

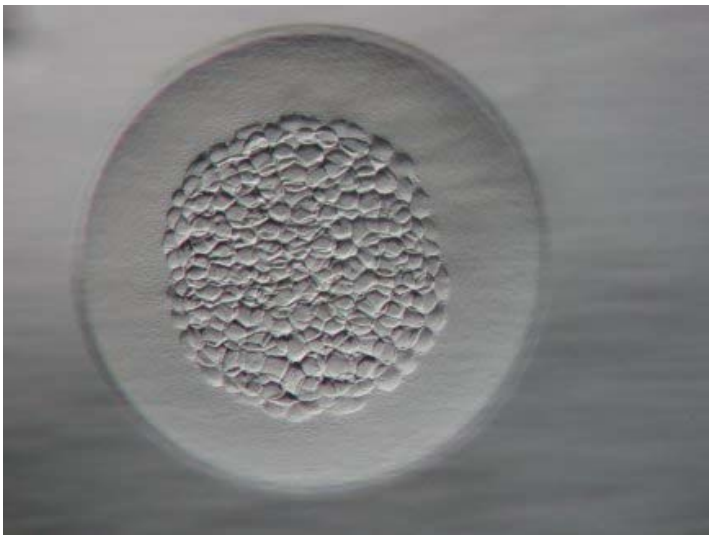

Figure 3. Sablefish egg at the late morula stage. This occurs at 7.6 °C-days.

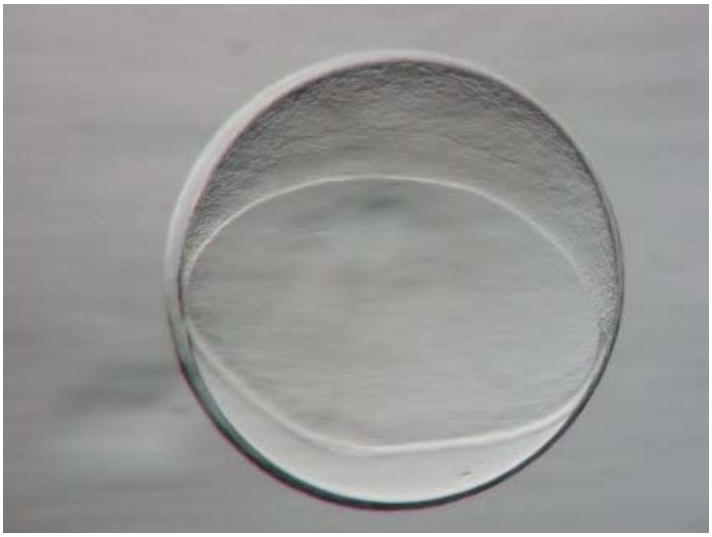

Figure 4. Sablefish egg at ½ to ¾ epiboly or yolk overgrowth. This occurs at 27.1 °C-days.

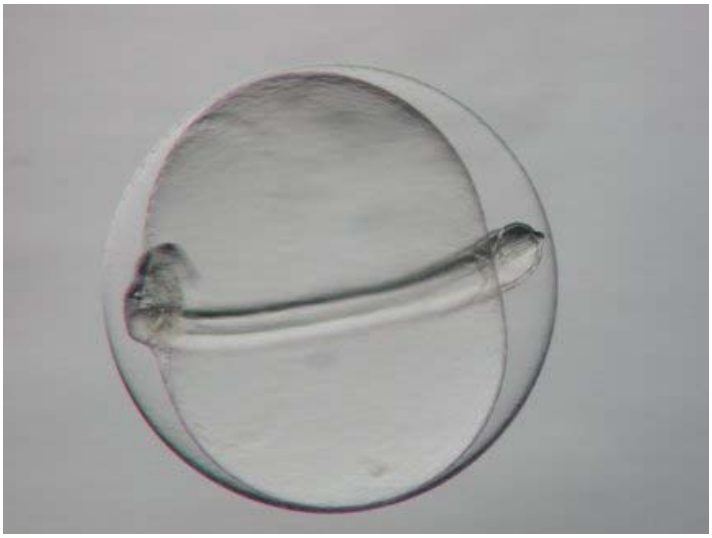

Figure 5. Sablefish egg with tailbud lifted off yolk. This occurs at 47.8 °C-days.

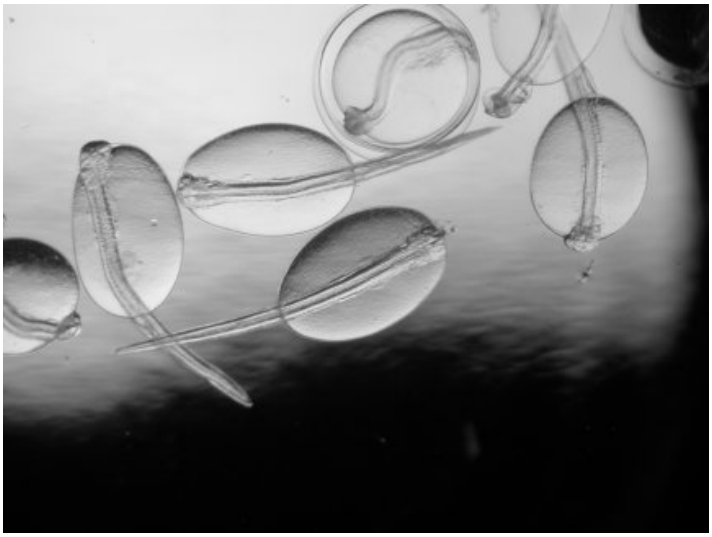

Figure 6. Hatched sablefish larvae. This occurs at 74.4 °C-days.

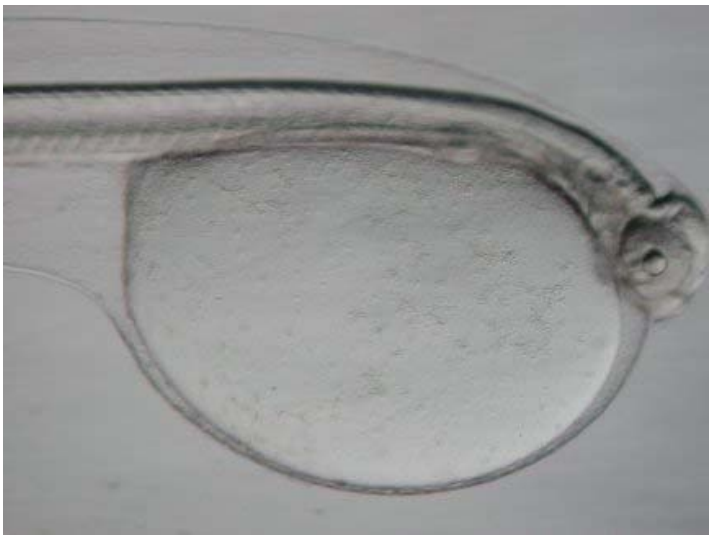

Figure 7. Sablefish larva with 50% yolk remaining. This occurs at 96.5 °C-days.

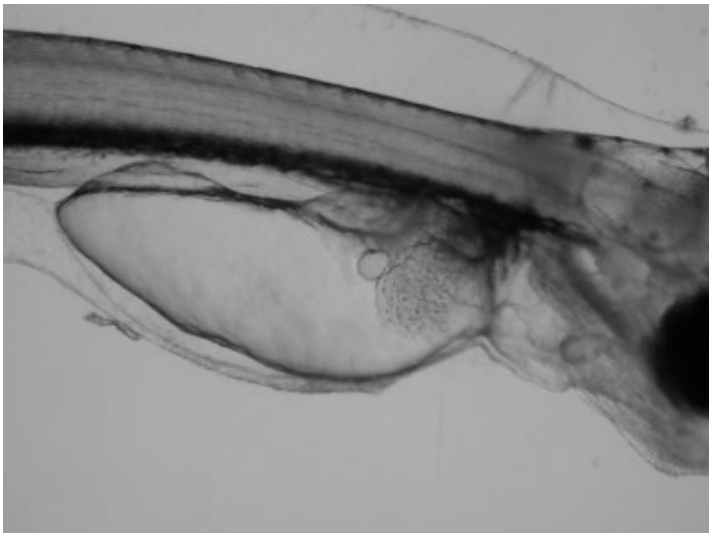

Figure 8. Sablefish larva near complete yolk absorption (about 2.5% yolk remaining). This occurs at 226 °C-days.

Table 1. Time (hr) and ATUs to various developmental stages of sablefish at temperatures ranging from 4 to 8 °C.
* Based on development at 6°C

Developmental stage	Time to stage (hr) 4°C	5°C	6°C	7°C	8°C	ATUs (°C-days)*
2-cell	7.1	5.9	5.3	4.8	4.6	1.3
4-cell	11.2	9.4	8.3	7.7	7.3	2.1
8-cell	15.7	13.1	11.6	10.7	10.2	2.9
16-cell	19.1	16.1	14.2	13.1	12.5	3.5
32-cell	23.6	19.8	17.5	16.2	15.4	4.4
64-cell	28.8	24.2	21.4	19.7	18.8	5.3
Late morula	41.0	34.4	30.4	28.1	26.7	7.6
Germ ring formation	84.5	70.9	62.7	57.9	55.1	15.7
Germ ring overgrowth beginning	108.3	91.0	80.4	74.2	70.6	20.1
1/4-1/2 epiboly	121.0	101.6	89.8	82.9	78.9	22.4
1/2-3/4 epiboly	146.0	122.6	108.3	100.0	95.2	27.1
Near yolk plug closure	181.3	152.2	134.5	124.1	118.2	33.6
Yolk plug closed	187.2	157.2	138.9	128.2	122.1	34.7
1-15 somites, kupffer's vesicle present	203.8	171.1	151.2	139.5	132.9	37.8
15-25 somites	222.8	187.0	165.3	152.5	145.3	41.3
>25 somites, optic cups developing	232.7	195.4	172.7	159.4	151.7	43.2
Tailbud lifted off yolk, otic vesicle, lens	257.6	216.2	191.1	176.4	167.9	47.8
Olfactory pits present	270.9	227.4	201.0	185.5	176.6	50.2
Tailbud extended, embryo near 1/2 circle	270.9	227.4	201.0	185.5	176.6	50.2
Embryo near 2/3-3/4 circle	308.7	259.2	229.1	211.4	201.3	57.3
Heart beating	332.6	279.2	246.8	227.8	216.9	61.7
Hatch	400.9	336.5	297.5	274.5	261.4	74.4
3.6 mm^3 ~75% yolk volume remaining	444.9	355.9	296.6	254.2	222.5	74.2
2.4 mm^3 ~50% yolk volume remaining	579.0	463.2	386.0	330.9	289.5	96.5
1.2 mm^3 ~25% yolk volume remaining	828.6	662.9	552.4	473.5	414.3	138.1
0.12 mm^3 ~2.5% yolk volume remaining	1356.0	1084.8	904.0	774.9	678.0	226.0

Concluding comment

The new pictures presented in this paper are an improvement in clarity over earlier pictures (Alderdice et al., 1988a). They are presented here to illustrate this improvement. In addition the time to the various developmental stages in Table 1 should prove useful to researchers and fish culturists alike.

Acknowledgements

We thank Dr. Peter Rombough for the loan of the photo-microscopy equipment. We also thank Dr. Craig Clarke, Dr. Gidon Minkoff, Mr. John Blackburn, and Mr. Yingyi Chen for providing the fertilised sablefish eggs.

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THE MANIPULATION OF MATURATION TIMING IN SABLEFISH BROODSTOCK USING MODIFIED PHOTOPERIOD REGIMES

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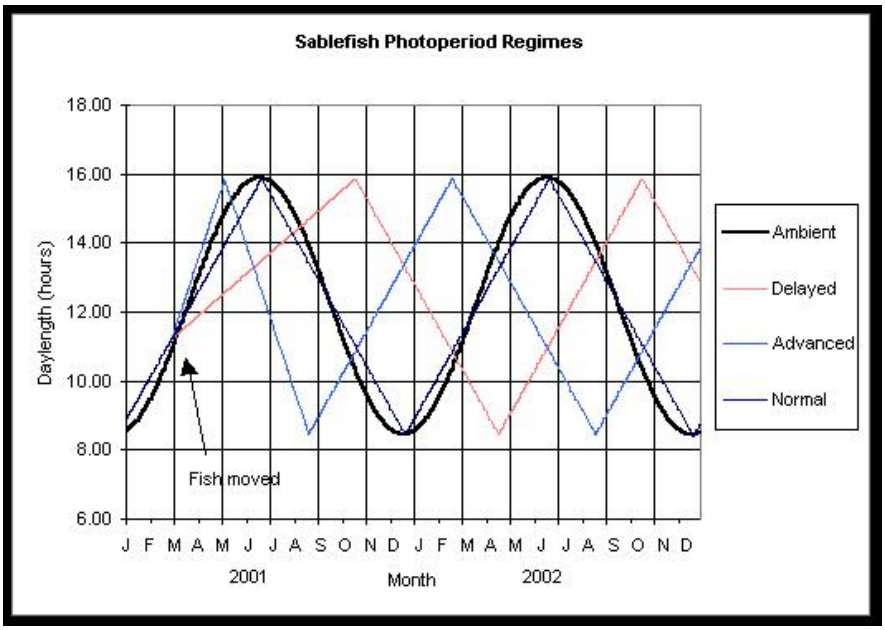
EXTENDED ABSTRACT ONLY - DO NOT CITE

The sablefish (*Anoplopoma fimbria*) has been identified as a priority species for aquaculture in the Pacific Northwest due to it's declining commercial and recreational fisheries, it's high value, as well as it's recognized potential for adapting well to captive aquaculture. In the wild, sablefish adults spawn in mid February at a temperature range of 4 - 6 °C, and approximately 700m depth. Prior research has indicated that holding captive broodstock in chilled sea water 6 - 7.5 °C improves the quality of gametes obtained from sablefish. The present study uses a chilled (constant 6 °C) recirculating seawater system coupled with computer controlled photoperiods to alter the timing of maturation in wild caught sablefish broodstock. Artificial control of maturation in this species will provide year-round production of gametes enabling the rapid development of techniques to optimize broodstock and larval rearing, which are typical bottlenecks to aquaculture.

Ninety six wild caught adult sablefish (of mixed sex), previously maintained in a single pass, shore based pumped seawater system (temperature range 8°C - 13°C) for over a year, were moved in March 2001 to a 15,000 gallon closed recirculating seawater system held at 6°C. The fish were distributed between three different photoperiod regimes designed to advance maturation by 4 months (n=21), delay by 4 months (n=21) or mimic the normal spawning time (n=56)

(Fig 1.). All fish were pit tagged so growth rate and response to the environmental conditioning could be monitored for each individual. Where possible fish maturity status and sex was determined by gonad biopsy, however, not all fish provided an adequate biopsy for such analysis. Fish were fed to satiation once per week (approximately 0.74% body weight on a wet weight basis).

Fig 1. Advanced, normal and delayed photoperiod regimes



Of the fish in the advanced group, 48% were identified as having maturing eggs in October-November, however only 10% of these proceeded to grow their oocytes to a size suitable for induced final maturation (1.1mm diameter) in December and January. The remaining fish reabsorbed their eggs; these fish remain on a phase shifted photoperiod and are expected to mature in fall 2002.

Of the fish in the normal photoperiod (synchronous with the ambient photoperiod), 72% of known females (from biopsies) reached an oocyte size suitable for implantation between February and May 2002. We have not detected any females in the normal photoperiod that have initiated oocyte development and subsequently reabsorbed oocytes prior to reaching a size suitable for spawning induction. The remaining 27% of females which have failed to mature in this cycle are expected to mature in the following cycle (Spring 2003). Several females in this group were lost due to a mechanical failure.

Fish in the delayed photoperiod group are expected to mature and spawn in June – July 2002. Identification of fish with developing oocytes in this group has not been completed at the time of abstract submission, but will be discussed in the poster.

Overall mortality to date has been 16%. All post spawned females have died within approximately a week after final egg stripping. Also, several implanted females have shown incomplete oocyte hydration and have died prior to releasing all of the mature oocytes in their ovaries. Mature females have, at this point, contributed 44% to the overall mortality rate.

Final maturation and hydration has been induced successfully with LHRHa (D-Ala^{6},Des-Gly^{10}Pro^{9}-LHRH, ethylamide), delivered by 95% cholesterol made at our lab. Two females have ovulated successfully without LHRHa implants, delivering a total of 265,000 eggs. Total egg take has reached 2.25 million eggs from 9 females, with an average of 250,000 eggs (approximately 160 eggs/ml). Eggs stripped per batch range from 16,000 - 289,000. The maximum egg take from a single female over time has been 535,000 eggs. Fertilization rates have ranged from 0-87%. This data shows that production can reach 465,000 fertilized eggs from a single female broodstock in this type of recirculating aquaculture system.

Initial results using LHRHa to induce spermiation in males does not appear to pose a problem, although sperm quality has not yet been investigated. While incubation to hatch has been achieved in initial trials, the construction of egg incubation facilities in our laboratory is not yet complete. Therefore, hatching rate and post-hatch survival have not been investigated. Incubation systems, however, are currently being developed for larval production.

Our results indicate that it is possible to use intensive state-of-the-art recirculation systems and photoperiod control to produce maturing male and female sablefish. Thus, the production of larvae may be possible, for supplying to researchers, and eventually commercial production systems on a year-round basis.

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PROBLEMS ENCOUNTERED

WHILE USING BAKING SODA SOLUTIONS

FOR RINSING SALMON EGGS

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Abstract

Pre-rinsing unfertilized eggs with a baking soda solution is an effective way of countering the harmful effects of ruptured egg material on fertilization rates but there are problems when using this procedure in large scale fish culture. With large batches of eggs it is difficult to thoroughly decant the final rinse solution so precise control of the amount of baking soda at fertilization is often impossible. Excess baking soda can result in sperm dilution and reduced fertilization. We found that egg mortality increased linearly from 5 % when the sperm dilution was low, to 24 % when the volume of baking soda was equal to the egg volume at fertilization.

Introduction

The presence of even small amounts of material from ruptured eggs interferes with sperm motility and results in low fertilitization rates during artificial insemination. Wilcox et al. (1984) found that the contents of just 8 eggs mixed with the ovarian fluid of 3000 eggs reduced sperm motility. In a test group with

1 % broken eggs the mortality was 60 % compared to a control mortality (0 broken eggs) of only 6 %. This is an important effect because eggs can easily be broken by rough handling or by improper use of stripping knives. Rinsing eggs with a solution of isotonic baking soda (13.68 grams/liter) prior to fertilization solves this problem (Wilcox et al. 1984).

This procedure is recommended where broken eggs are suspected or when fertilizing extremely valuable stocks. Although this pre-fertilization rinse is simple and effective, we have encountered some problems during large-scale fish culture operations that can lead to increased mortality.

On the other hand rinsing with baking soda is sometimes performed after fertilization in order to remove excess sperm and blood. This procedure is presumed to reduce fungus growth during incubation. Although this post-fertilization rinse has fewer complications, it does not prevent broken egg material from blocking fertilization.

Materials and Methods

The recommended method for countering the effect of broken eggs is to double rinse the unfertilized eggs in 2.5 times their volume with isotonic baking soda solution ($NaHCO_3$, 13.68 g/l). After decanting the rinse solution, fresh solution, amounting to 25 % of the egg volume, and sperm are added without delay (Wilcox et al. 1984).

We observed that in production-scale fish culture where large egg batches are handled, decanting the baking soda solution in the final rinse was difficult to control. It often resulted in a final volume of solution that was more than 25 % of the egg volume. This greater volume would lead to sperm dilution and possibly reduced fertilization (Billard and Jensen, 1996). To test this possibility, we fertilized batches of eggs containing final volumes of $NaHCO_3$ amounting to 0%, 25 %, 50% and 100% of the egg volume. Treatments consisting of a post-fertilization rinse and no rinse (control) were also tested.

Chum salmon from the Big Qualicum Hatchery were used in the study. Eggs from 12 females and milt from 7 males were pooled to reduce gamete differences. There was no signs of broken eggs in the pooled group. Each treatment consisted of approximately 500 eggs (130 ml) and was replicated three times. Using the 25% treatment as an example, the sequence of events was as follows: (a) measure 130 ml of eggs into a container (b) rinse twice with 2.5

times the volume of $NaHCO_3$ (325 ml) (c) decant completely and add 32.5 ml (25%) of fresh $NaHCO_3$ (d) fertilize with 2 ml of sperm (e) mix and wait 30 seconds (f) pour into the Heath incubation tray for water activation of the eggs and the start of development. The other treatments were identical except that after the final decant, the volumes of fresh $NaHCO_3$ added were 0 ml (0%), 65 ml (50%) and 130 ml (100%). The final decant was standardized by fixing a net across the mouth of the egg bucket and inverting for 10 seconds. In the post-fertilization rinse treatment, eggs were fertilized, rinsed twice with NaHCO3 and poured into the incubator. For the control, eggs and sperm were mixed and poured into the tray after 30 seconds.

Eggs were fertilized on Nov 13, 1998. Incubation trays were observed and the dead eggs were removed and counted on Jan 5 (post-eyed), Jan 8, Jan 25 and March 10, 1999. Live eggs were also counted on Jan 8 so that the exact number of eggs in each treatment was known. Water flow through the Heath stacks was 12 liters/min.

Finally, sperm concentration of the pooled milt was determined using a haemocytometer.

Results and Discussion

Egg mortality from fertilization to ponding for the six experimental treatments showed significant differences (at the 0.05 level) between treatments (F = 14.4, critical F = 3.1, df = 5, 12).

Table 1. Egg mortality (%) for each treatment. Pre-0%, 25%, 50%, 100% indicate the volume of $NaHCO_3$ at fertilization (as % of egg volume) after the pre-fertilization rinse.

	Control	Post-Fert	Pre-0%	Pre-25%	Pre-50%	Pre-100%
Rep 1	7.2	11.6	4.1	6.8	17.3	24.9
Rep 2	11.4	11.2	4.5	8.7	11.4	19.9
Rep 3	7.2	5.1	6.3	7.3	8.2	27.4
Mean	**8.60**	**9.33**	**4.94**	**7.57**	**12.33**	**24.08**

The treatment with the greatest volume of $NaHCO_3$ at fertilization (Rinse 100%) had the highest mortality. Most of the dead eggs were removed on Jan 5 and Jan 8, 1999 and showed no signs of development. Hence, for these eggs it is

presumed that sperm were too diluted in concentration and that fertilization did not occur.

Regression analysis showed that that there was a significant relationship between egg mortality (Y) and the amount of baking soda at fertilization (X) (Fig. 1). The least squares trend line in Figure 1 is given by: $Y = 3.6361 + 0.1964X$ ($r^2 = 0.875$).

Figure 1 shows that failure to control the final volume of baking soda at fertilization can lead to increased mortality.

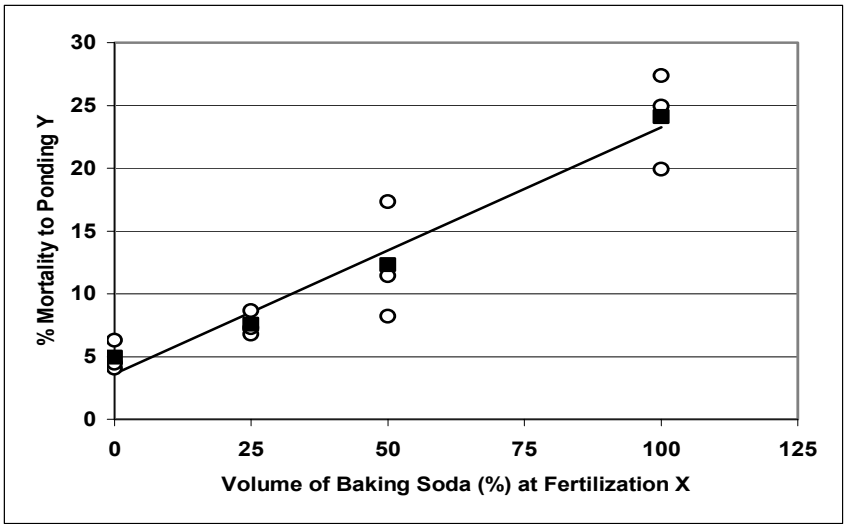

Figure 1. Egg mortality (%) Y versus volume of baking soda at fertilization (as % of egg volume) X. Replicates, treatment means (solid squares) and the trend line are also shown. The equation for the linear relationship is $Y = 3.6361 + 0.1964\ X$; r^2=0.875, F=70.2 (critical F=4.96, df=1,10 at 0.05).

To see how this could happen during large scale fish culture consider the following example where the pre-fertilization rinse procedure is being used on a 1 liter batch of eggs. During the final rinse 2.5 liters of $NaHCO_3$ is used. If the final decant is performed simply by tipping the bucket on the floor, it is conceivable that a considerable volume of $NaHCO_3$ is left in the egg bucket when the final 0.25 liters of fresh $NaHCO_3$ is added just prior to fertilization. If

2 liters was removed in the final decant so that 0.5 liters was left in the egg mass, the final volume after addition of 0.25 liters of fresh $NaHCO_3$ would be 0.75 liters (75%). With the protocols used in this experiment (2 ml milt per 500 eggs, 30 second contact time), this procedure would cause significant increase mortality (Fig. 1). Using baking soda to counter the effects of broken eggs requires careful decanting and accurate volume measurements so that the final volume of $NaHCO_3$ is no more than 25% of the egg volume.

The sperm concentration from the pooled sample was found to be 26 $x10^9$ per ml. Hence, egg mortality, when plotted against sperm concentration (Fig. 2) in the various final rinse volumes resulted in the following curvilinear relationship: $Y = 5.1993811 + 1.5344362e+14/X^{1.5}$ ($r^2 = 0.8927$). This translates to 16 million sperm per egg for the treatments reported herein, which is greater than the 0.5 to 1.0 million sperm per egg concentration suggested by Billard (1992) as being adequate for successful fertilization. Hence, other factors such as sperm concentration, temperature, and contact time prior to water activation likely interact to affect fertilization success. Given the conditions in our tests, egg fertilization was noticeably reduced in all rinse treatments compared to the control.

Another potential problem with this method can occur with the warming of the baking soda solution. Large volumes must be prepared and stored over several hours during egg takes. Depending on the initial water temperature and air temperature the solution can warm to lethal levels. On warm days it may be necessary to store the baking soda in a flowing water bath. Temperature should be monitored and the solution discarded if it is significantly warmer than the incubation water supply.

This procedure should be tested on a production scale before being fully implemented at a hatchery. Practical procedures must be established for: (a) preparing and handling large volumes of baking soda (b) decanting the rinse solution (c) control of the final $NaHCO_3$ volume at fertilization. These new procedures will require more manpower and will increase handling times. To avoid problems and reap the benefits of this very useful technique, careful planning and production scale testing are required.

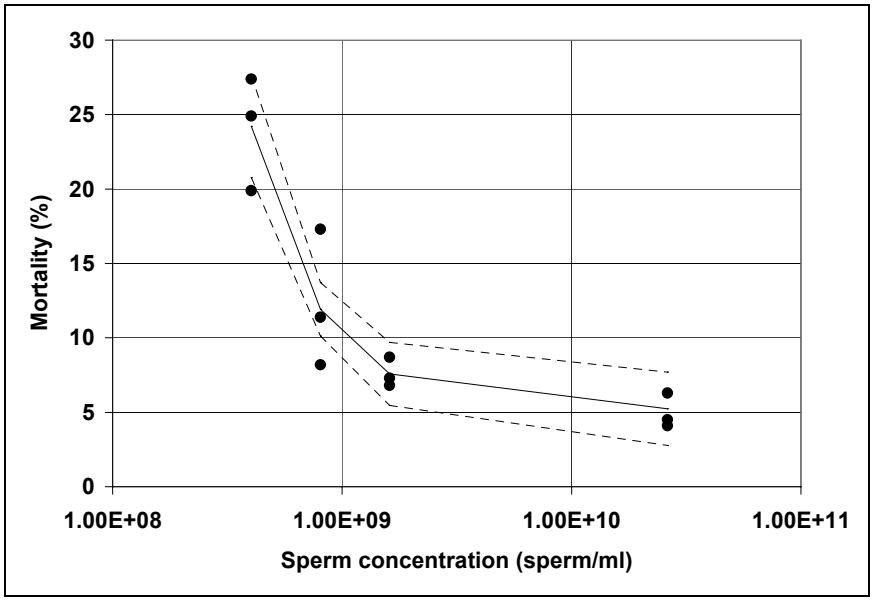

Figure 2. Egg mortality (%) versus sperm concentration (sperm/ml). The equation for the curvilinear relationship is y = 5.199381 + 1.534436e+14/$x^{1.5}$; r^2=0.8927, F=83.2 (critical F=4.96, df=1,10 at 0.05). The replicates (solid circles), the curvilinear line (solid line) anf 95% confidence limits (dotted lines) are shown.

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THE EMBRYONIC AND LARVAL DEVELOPMENT OF THE PIPEFISH NEROPHIS LUMBRICIFORMIS (PISCES; SYNGNATHIDAE).

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Syngnathids (seahorses and pipefishes) exhibit some of the most specialized forms of parental care in animals and so are ideally suited to the study of the evolution of parental care. Accumulating evidence suggest that environmental variables as well as anatomical and physiological constraints may strongly influence differences in potential reproductive rates between the sexes thereby influencing mate competition and ultimately sexual selection. Nevertheless, the number of publications regarding syngnathid embryonic and larval development is still scarce, therefore not allowing significant comparative work. In this work, some brief descriptions on the reproduction as well as embryonic and larval development of the pipefish *Nerophis lumbriciformis* (Jenyns, 1835) are summarily described. *N. lumbriciformis* is a small and slender pipefish, commonly found in the rocky intertidal to about 30m. During the breeding season, males brood their offspring attached to their flattened ventral surface (Mean number of eggs= 49; N=114; range=18-84; average=48.71; SD=10.84) and throughout gestation parental care is exclusively paternal. The courtship

behaviour of *N. lumbriciformis* consists of three distinct phases (initial courtship, spawning and embrace) marked by prominent behavioural changes. Nevertheless, unlike other syngnathids that rise during and/or after egg deposition, the entire courtship ritual takes place in close contact with the substratum. We suggest that, at the behaviour level, the reduction of vertical and swimming elements may constitute an adaptation to the typical intertidal physical conditions. After fertilization, the larval development lasted approximately 25 to 30 days (15-16 ºC and 14ºC, respectively). After hatching, the newborn juveniles are free-swimming and no further care is provided. In normal conditions, at the time of "birth" the yolk sac has completely disappeared. Nevertheless, if the males are confronted with a stressful event, the premature release of larvae still possessing the yolk sac might occur.

INFLUENCE OF EGG COMPOSITION ON FERTILIZATION RATE IN SABLEFISH, *ANOPLOPOMA FIMBRIA*

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Rearing technology is being developed so that the sablefish (*Anoplopoma fimbria*) can be introduced into intensive aquaculture (Clarke et al., 1999). In order for this to become a reality it is necessary to have a reliable supply of juveniles for grow out. Egg batches from captive sablefish exhibit highly variable rates of fertilization and hatching. Although there is scant information regarding egg composition in sablefish, it has been found that constituents in eggs from captive halibut broodstock show considerable variation (Nortvedt et al., 2001). The present study was undertaken to examine important components in relation to fertilization rate in captive broodstock. It is expected that

identification of constituents that correlate with egg fertilization will assist efforts to improve egg quality and thus, juvenile production.

Eggs were stripped from sablefish held at the Pacific Biological Station. Subsamples of unfertilized eggs were taken from each batch for analysis of fatty acids, free amino acids, minerals and vitamins. The remaining eggs were fertilized and egg viability was assessed.

Egg fertilization rates for 29 batches of eggs varied from 1.7% to 95%. Correlations between the level of selected egg components and the percentage fertilization rate for the corresponding batches are shown in table 1.

Table 1. Correlation coefficients between egg components and % fertilization rate (bold font indicates statistically significant at $p<0.05$ level).

Component	**r**	**Probability level of correlation**
Alanine	**0.46**	0.021
Arachidonic acid	-0.19	0.350
Asparagine	**0.45**	0.024
Aspartic acid	**0.45**	0.023
Docosahexaenoic acid	0.06	0.778
Eicosapentaenoic acid	-0.07	0.745
Folate	**0.49**	0.012
Glutamine	**0.41**	0.039
Glycine	**0.55**	0.005
Histidine	**0.54**	0.005
Isoleucine	0.38	0.059
Leucine	**0.45**	0.025
Lysine	**0.56**	0.003
Methionine	**0.42**	0.034
Phenylalanine	**0.46**	0.021
Selenium	-0.32	0.158
Serine	**0.47**	0.017
Taurine	**0.49**	0.013
Valine	0.38	0.058
Vitamin C	0.19	0.344
Vitamin E	-0.02	0.932

There were significant positive correlations among many of the free amino acid concentrations (mg/g wet tissue) and fertilization rate. However fertilization rate was not significantly correlated with fatty acid or mineral concentrations. Of the vitamins examined only the B vitamin folate had a significant positive correlation with egg fertilization.

The consistent relationship between fertilization rate and free amino acid levels may reflect the importance of the latter as osmolytes and substrates in energy metabolism of eggs and embryos (Rønnestad and Fyhn, 1993).

Acknowledgements

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THE EFFECTS OF WATER TEMPERATURE AND OTHER GROUNDWATER VARIABLES ON THE INCUBATION SUCCESS AND EARLY EMBRYONIC GROWTH OF BULL TROUT, IN PRISTINE ROCKY MOUNTAIN STREAMS.

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Groundwater has been frequently cited as an important determinant of bull trout spawning site selection and incubation success. Bull trout can be highly selective when choosing spawning locations and spawning redds are often located in specific stream reaches and even micro-sites that exhibit high surface water and groundwater exchange (Baxter & Hauer, 2000). Bull trout incubating in areas with substantial subsurface flow can benefit from the high oxygen content of the surface water and the thermal stability of the groundwater (Baxter & McPhail, 1999). Previous work in the Williston Lake Watershed, British Columbia, has identified geographically separated bull trout spawning sites that show unique, stable thermal signatures throughout the year. The importance of the yearly temperature regime for the development of early life stages is not known. It is also not known how small deviations from the thermal and

chemical regime characteristic of bull trout spawning habitat may affect spawning site selection, incubation success and ultimately juvenile survival. We therefore examined the influence of inter-gravel temperature, pH, conductivity, inter-gravel flow, and oxygen on bull trout survival, development, embryonic growth and yolk utilization. We completed assays of incubation success and growth using fertilized bull trout eggs contained within perforated plastic capsules. The capsules were buried at typical redd depths in three spawning tributaries draining into the Williston Reservoir, B.C. Eggs were also incubated in a temperature controlled laboratory environment to assess growth and timing of developmental stages under natural and two elevated thermal regimes (+1.5 ^{o}C and +3 ^{o}C).

The alevins pictured in Figure 1 were collected from the laboratory experiment on March 18, 2002, near the time of button-up (+3 ^{o}C regime) and 50% hatch (natural regime). Substantial differences in alevin length and yolk utilization rates were evident between temperature replicates within both the field and laboratory experiments. We observed a difference in length of more than 10 mm between the control and warmest experimental groups in the laboratory (Figure 1). The timing of hatch, button-up as well as growth and yolk utilization were all advanced relative to the control temperature for both of the experimental temperature replicates (Table 1). Fifty percent hatch was advanced by 56 and 77 days for the experimental mid temperature and warm temperature groups, respectively, compared to the control group. In both of the warmer temperature replicates button-up occurred approximately 3 months following fertilization. An example of the effect of temperature elevation on rate of development is seen in the time of button-up compared to hatch. Growth and development of the warmest temperature replicate was so advanced that hatch of the control fish occurred within 9 days of button-up for the warmest group (Table 1).

Figure 1. Bull trout collected from each of three experimental temperature regimes.

Table 1. Date, number of days post fertilization, and Accumulated Thermal Units (ATU) for 50% hatch and button up. ATU is calculated by summing the average temperature for each day over the duration of the measured interval.

	Simulated Natural Regime	Elevated by 1.5 °C Regime	Elevated by 3.0 °C Regime
50% Hatch			
Date	Mar 17, 2002	Jan 20, 2002	Dec 30, 2001
# days post fertilization	188	132	111
ATU	271	437	472
Button Up			
Date		Apr 23, 2002	Mar 27, 2002
# days post fertilization		224	197
ATU		604	768

Another striking result of our study is the length of time required for development to hatch and button-up in these northern populations of bull trout. Using an Accumulated Thermal Unit curve (ATU, measured in degree days) developed by Gould (1987) from a population of Oregon, USA bull trout, we calculated the time for hatch of our stock to be approximately three weeks earlier than we actually observed. This indicates that northern populations appear to develop more slowly than southern populations. Our results also demonstrate an interesting relationship between ATU and time for hatch and button-up (Table 1). The number of degree-days to achieve a specific developmental stage decreases with temperature. In the warmest temperature group, 768 degree-days were required for complete button-up. In the mid temperature group, 604 degree days were required for complete button-up. Thus, the relationship between ATU and development is not constant for larval development at very low temperatures.

Our results confirm and expand on existing work examining the effect of temperature on larval development in salmonids. For bull trout, even apparently small differences in incubation temperature can have substantial impacts on spawning success. As shown by previous researchers (see Rombough 1997) early developmental stages in fish are particularly sensitive to temperature changes, with the acceleration of development in salmonids being especially dramatic. Alterations in the stream environment related to industrial development or changes in global climate can cause stream warming or changes in groundwater flow patterns. The resulting changes may cause precocial exhaustion of yolk reserves and early emergence with immediate negative ecological consequences for bull trout in cold northern streams.

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COMPARATIVE STUDY OF QUALITY AND QUANTITY OF SEMINAL FLUID IN CULTURED SALMONIDS OF IRAN

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Introduction

Rainbow trout scientifically called, *Oncorhynchus mykiss*, is one of the cultured fishes in cold and more fresh waters of the world. Its culture in Iran goes back to forty years ago which it was first done, through importing eyed eggs and was bred in several of private and governmental centers .A lot of researches have been done in Iran on this species which mainly focused over the female fish. In the world some scientists such as Billard & Cosson have done study on reproduction in rainbow trout, sex differentiation, dynamics of gametogenesis, biology and preservation of gametes Aquaculture. Also Ginzburg has written one book about polyspermy in fishes, So Hultz has researched about Cryopreservation and so on..
This research is the first classic one on male broodstocks of rainbow trout.

Aims

In this project, the main objective was qualitative &quantitative evaluation of semen in two years old spawners of 3 phenotypes which could be found in Iran and finally to determine effect of different factors , their influencing on fertilization process and percentage.
This task accompanished from the beginning of the reproductive cycle up to the end in one of the advanced Aquaculture centers of trout in the country (Karaj Mahi Sarai).
Other objectives of this research were;

- Identify more accurately the present phenotypes of male broodstocks
- Identification of the exact time of breeding.

Experimental Procedures

A- In order to identify the three phenotypes namely,
1. UNCLEAR
2. CLEAR
3. CLEAR WITH ORANGE BANDS

were compared. The factors, length, width, thickness, weight of body, weight of gonad, GSI, sperm motility, total volume and concentration of sperm were measured as the major characteristics of phenotypes in this fish.

B- In the second experiment, a randomized complete block design was run in two different periods of time (21days intervals).In each incubator an artificial insemination was run on eggs from different female broodstocks which was divided into 15 blocks having three rows and five columns ,so as the columns represent fish from different phenotypes & the rows indicate the changes in sperm volume from each phenotype.
In each period following insemination with different volume of sperm (0.1ml to 0.3 ml for each 500 ova from several brood female fish) changes occurred in the percentage of fertilization in different
phenotypes.

C- In the third stage of experiment, the aim was to compare the effect of each phenotype sperm to a mixture of sperms of all phenotypes on ova, which mimics the traditional procedure.

D- In the 4^{th} stage, the insemination was done with application of a two factor, factorial design, with time as factor A, phenotype as factor B and different volumes of 0.1,0.2 &0.3 ml as replications.

E- The last part of the research was to obtain the factors influencing percentage of fertility.

F- Along with the above experiment, and after staining the sperms with eosin, the length of spermatozoid, length and width of spermatozoid head was measured and recorded.

Conclusion & Discussion Summary

The results of above experiments is respectively;

A- These measurements proved that male broodstocks are of three different phenotypes in their body weight ,sperm motility and concentration,($p<0.05$) but statistical difference.

B- The results showed that in the first period phenotype-3 (clear with orange band) was superior than the other two (P<0.05) and in the second period phenotype-2 (clear) showed superiority to the other two (P<).o1). During this experiment the percentage of infertility and mortality of the eggs was also measured.
For different volumes of sperm ,a Duncan Multiple Range Test(D.M.R.T) was run and the).1 ml volume of sperm was put in one group and the other group volumes assigned to the other two groups(P<0.05).

C- The results showed that only the phenotype-1 was inferior(P<0.01) relative to the other treatments. Some experts believe this population isn't safety and there are some reasons for unclear skin in addition to originate such as ecological factors (high temperature ,low oxygen, etc) ,stress, misfeeding, low healthy ,diseases, and so on.

D- For the first period the average percentage of fertility was 82.44 and second period was 67.93. also the fertility was in the order of phenotype-2, then 3 and 1,respectively.The interaction of the time and
phenotype showed a consider fall in percent of fertility for the phenotypes-3 during the second period.

E- with the assumption that in all treatments, the qualitative factors of the eggs kept the same, and the only fluctuation in fertility was by different sperms, factors responsible for such fluctuation can be classified as, volume of sperm used to fertilize , density of sperm, sperm motility and number of spermatozoa used for one ovum and GSI.
Considering all above factors, a regression equation was developed with an R2 =0.82

F- The values are as follows;

Total length of spermatozoid = 41.25+/- 1.5 micron
Length of Spz Head=4.25+/- 0.25 micron
Width of Spz Head = 3.5 +/- 0.2 micron

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CHILLED INCUBATION PROVIDES IMPROVED SURVIVAL FOR COHO SALMON

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Introduction

The Inch Creek Salmon Hatchery was built in 1982 to provide incubation and rearing capacity to help support local stocks of coho and chum salmon. The well water supply at Inch Creek Hatchery has a reverse temperature profile, making it warm (up to 13° C) in the winter and cool (down to 5° C) in the summer (Figure 1).

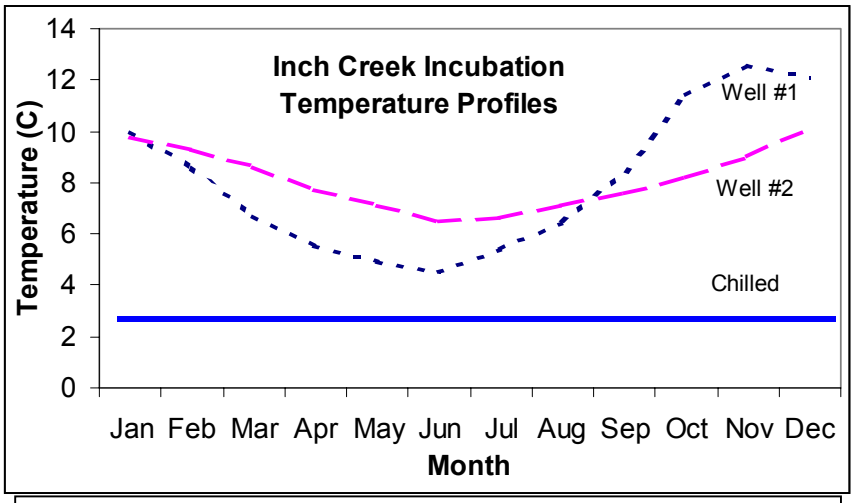


Figure 1. Annual temperature profile for Inch Creek Hatchery wells.

Two major problems when culturing coho have been encountered at this facility:

1. The warm water incubation causes an early ponding and very long rearing period for the fish, requiring a low ration level to meet a target release size of 20 grams. We do not consider near-starvation rearing to be good for the fish.
2. Periodic myxobacterial infections have occurred, usually in April and again in July. The virulence of these infections has been variable from year to year, stock to stock, and from container to container within the same stock and year. The infections required egg targets to be inflated by 20-30% to compensate for rearing mortality.

Delaying the onset of ponding would allow a higher ration feeding regime and might avoid the seasonal onset of myxobacterial infections. In 1994 we conducted a small-scale test on about 10% of our egg target to delay fry ponding by chilling incubation water to 4^{o} C. In 1995 we renovated our incubation room to chill the water to 2^{o} C for 30% of production. In 1998 we made further changes to incubate all coho (1 million eggs) on chilled water.

Methods

The chilled / recirculated incubation consists of a standard Heath tray system modified with the following components:

1. Pump: a 3 hp electric pump circulates about 400 Lpm flow through the system. Electrical power to the pump is backed by the hatchery emergency generator system.

2. Chillers: two 2 hp units and two 3 hp units in series with capacity to cool 400 litres / minute from 14^{o} C to 2^{o} C. An ultra violet sterilization tube treats ½ of the process water on each circuit.

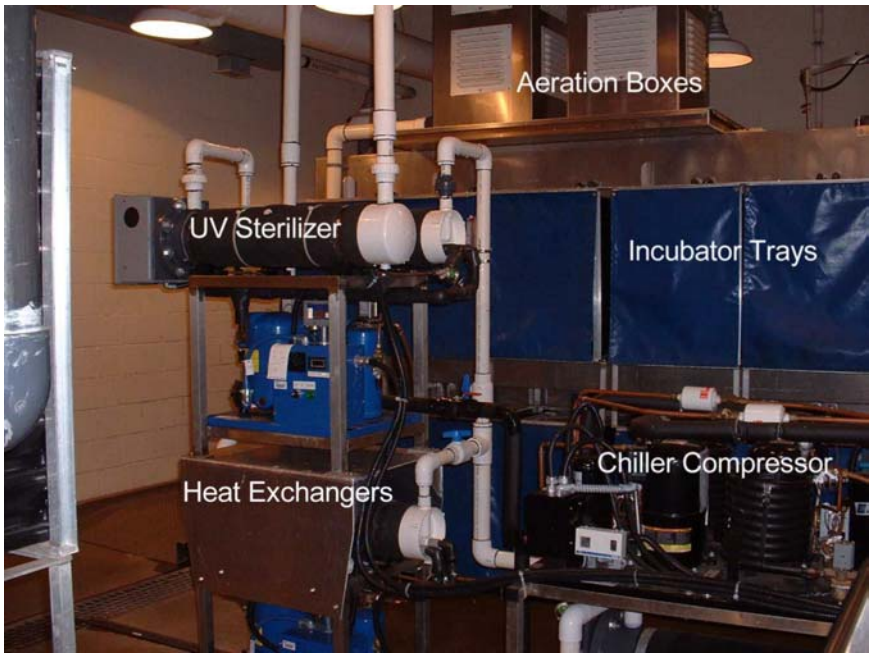
3. Aeration: two boxes, one each for the upper and lower banks of Heath trays. Each box consists of a calibrated distribution plate, a media bed of 1.5 inch diameter flexi-rings, and louvred walls to allow ventilation. This type of media filled box can also act as a biological filter for ammonia.

4. Incubators: Heath type incubation trays arranged in stacks of 8 trays, 10 stacks on an upper level in two rows and 10 stacks on a lower level in two

rows. Each stack is set to a flow of approximately 15 litres / minute and loaded with 7-10,000 coho eggs.

5. Sump: a 500 litre polypropylene box sunk in the incubation room floor. The sump box collects discharge from the Heath trays and acts as reservoir for the pump. Fresh make-up water, about 40 lpm, is added to the sump, and overflow from the system discharges over the lip of the sump tank directly to floor drains.

Photograph 1. Chilled water system at Inch Creek Hatchery.

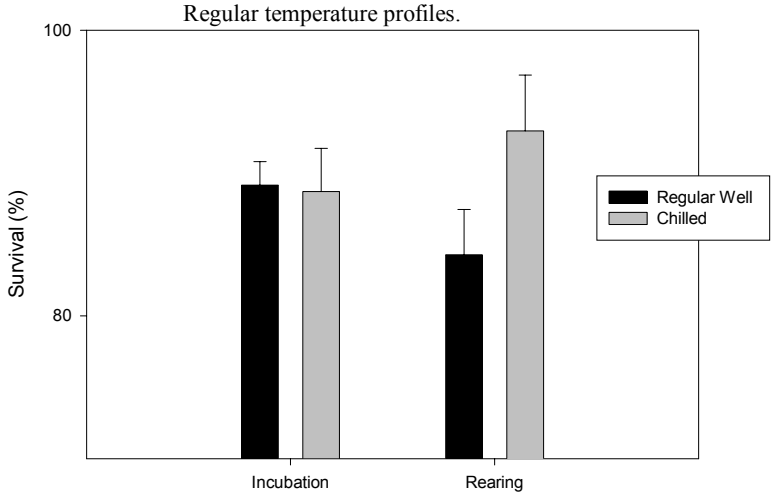


Results and Discussion

Incubation

Initially we were concerned that a prolonged incubation period in cold water could kill fish and reduce fry quality (Weatherly and Gill, 1995) or that a build-up of nitrogenous wastes in the incubators could have detrimental effects during incubation or subsequent rearing (Sigma, 1985). However, there has been no

Figure 1. Survival of fish incubated in Chilled versus Regular temperature profiles.



noticeable difference in overall incubation survival (Figure 1) for chiller versus regular well groups of coho; a mean of 89% from green egg to ponding for both groups. Nitrate and nitrite have remained well below toxic levels, perhaps due to the build-up of nitrifying bacteria on the aeration media.

Although wild coho often withstand cold incubation temperatures, Weatherly and Gill (1995) recommended an initial incubation requirement of 6 days at 6^{o} C

(up to the blastula stage of development). This would have limited our flexibility to manipulate ponding dates. Since Inch egg-takes occur from mid November to late December we needed to keep the chiller system adjusted to 6^{o} C until 6 days after the last egg-take. We have experimented with various groups to test the 6^{o} requirement with mixed results (Table 2). In most cases initial incubation at 6^{o} C gives the best survival.

Rearing

We have had no difficulty raising coho to the target release of 20 grams in mid May. We encountered a problem with pinheads in the first (1994 BY) trial group and had a systemic myxobacteriosis infection in a single channel of 1997 BY Inch coho. Otherwise all chiller incubation groups to date have been disease free. The mean rearing survival of chiller groups has been 93% compared to 84% for regular well groups (Figure 2).

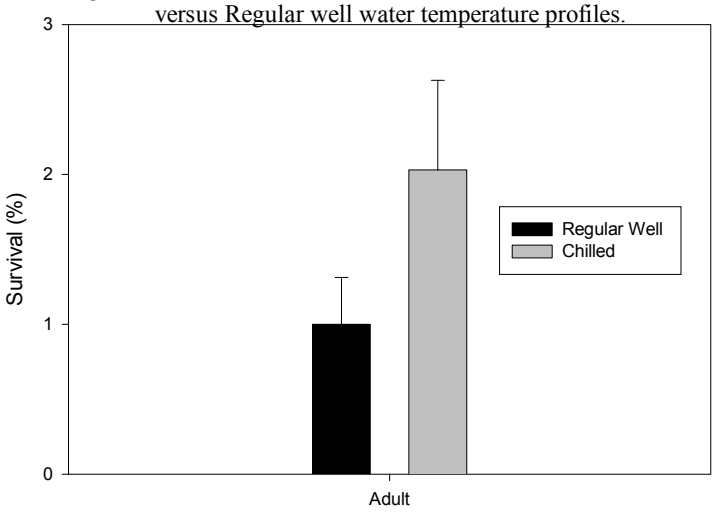
Use of the chiller has led to a major change in hatchery operation. In the past, 1 million coho were ponded in intermediate rearing troughs (IRTs) or circular tubs by mid February and required daily maintenance. These fish were normally 2 grams in size (reaching densities of 25 kg/m$^{3)}$ before release of the previous brood year's coho allowed transfer to concrete raceways. Now those 1 million fish remain in the incubation room an additional 5 months with minimal attention required. Chiller groups are transferred from IRTs to raceways in August at less than 1 gram and before densities reach 10 kg/m^{3}.

The low stress rearing conditions and higher ration level probably account for the reduced rearing mortality. We have adult survival results from 3 brood years of chilled Inch coho. These groups were marked with coded wire tags (CWTs) and released under nearly identical final rearing conditions. The chiller fish survived at twice the rate of regular well groups (Table 1).

Ocean Survival

The survival from smolt to adult of two brood years of coho from Inch Creek showed that the group incubated on chilled water survived at about twice the rate compared to fish incubated on the regular well water temperature profile (Figure 3.). This result should be taken with considerable caution, due to the large error associated with coded-wire tag returns.

Figure 3. Survival from Smolt to Adult of fish incubated in Chilled versus Regular well water temperature profiles.



Conclusions

In summary the use of chilled/recirculated water for coho incubation at Inch Creek Project has the following benefits:

- Equivalent incubation success to regular well water.
- Reduced rearing mortality.
- Reduced rearing program work load.
- Improved smolt quality.

The hatchery is supplied by wells that provide water of excellent quality but whose temperature profile is out of phase with surface waters. The well water can be as high as 13.5°C in the winter and as low as 5°C in the summer. This poses a problem for coho salmon culture because the hatchery's goal is to release coho smolts in the spring to mimic the natural life history of the stocks. Such high winter temperatures accelerate the development of eggs during incubation, such that the fish emerge prepared to feed in January, rather than in April when they would emerge in their natal streams.

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