Aspects of feeding, maturation and osmoregulation in cultured juvenile greenback flounder (*Rhombosolea tapirina*)

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ORIGINAL OBJECTIVES:

1. Identify the stage(s) during flounder larval/juvenile development when deformities occur.
2. Determine the effect of nutritional feed supplementation and environment on the deformities in the juvenile fish.
3. Establish a diel feeding profile for juvenile flounder, particularly in relation to light and dark cycles.
4. Measure the changes in blood osmolality during exposure to various salinity levels.
5. Construct a profile of maturation and relate this to water temperature and growth.
6. Produce juveniles for grow-out trials (as a consequence of producing fish for experimentation).

1. NON-TECHNICAL SUMMARY

Deformities
Skeletal deformities have been observed in cultured flounder in recent years. At times, high percentages of fish are affected, potentially influencing growth and marketability, and affecting the reliability of the experimental data collected. Many growers taking fish for pilot scale grow-out trials reported mortality and low growth in many fish with gross skeletal deformities. Although skeletal (spinal) deformities are of most concern, mal-pigmentation or pseudo-albinism is also significant. It is likely that nutritional deficiencies/imbalance are responsible for these conditions but as the nutritional requirements of this species are unknown, the causes are unidentified. Deformities in the past have been observed in fish larger than 5-10 g. It was the aim of this study to identify the timing of the appearance of the skeletal deformities during a documented larval/juvenile rearing period and to trial alternative production protocols. It was outside the scope of this study to identify the causes of the deformities. The results show that deformities are visible after a period of time on artificial diets, during the post-weaning period. Deformities were not evident during the live feed period.

The main objective in the detection of the spinal deformities was achieved but the manipulation of diet and environment was only achieved on a superficial level. Future work in this area would need to consider the complex interactions between temperature, live feed enrichment, weaning and artificial diet composition.

Feeding profiles
Feeds and feeding normally comprise between 40-60% of the production costs in fish culture. Optimisation of feed usage and growth (and FCR) minimises these costs and maximises production efficiency. Only one published paper (Burel et al., 1997) has been found on flatfish feeding profiles or rhythms with most work based on salmonids, goldfish and marine fish such as European sea bass. Greenback flounder is a stomachless flatfish which feeds in the wild on invertebrates rather than fish, preferring to feed during the day but can feed at night, although at a lower level (Crawford, 1984). They are also relatively slow, unco-ordinated feeders, which tend not to consume discrete meals as quickly as do the salmonids. Identifying the preferential feeding times and duration will provide a better understanding of the feeding behaviour under culture conditions in this species, which could in turn lead to the development of methods to optimise feed delivery and feed ingestion (and hence growth).

The results show that flounder juveniles approximately 50-150 g in weight prefer to feed during the day (light phase) except if hungry. Following a period of feed deprivation, fish tend to feed during the light and dark phases (night and day). This suggests that normal day feed delivery is possibly adequate for flounder as long as the delivery approaches satiation. As flounder feed during both light and dark phases, they probably use both visual and olfactory senses to locate pellets, as has been found in other flatfish species.

Osmoregulation
The aim of this section was to measure the changes in blood osmolality after direct transfer to various salinities. Few references are available on the osmoregulatory capabilities of greenback flounder. Other flatfish such as turbot and halibut have displayed better growth when cultured at lower salinities than seawater. Identifying the salinity tolerances of the fish also extends the possible range of sites for farming from marine to brackish (estuarine) and hypersaline (inland ground water, salt fields). Before quantifying the growth potential of fish in different salinities (outside the scope of this study), the responses of flounder to different salinities needs to be determined. This trial demonstrated that short term tolerances (as indicated by blood osmolality) to direct transfer only fell slightly in salinities down to 3 ‰ and rose slightly in 40 ‰. Freshwater was the only salinity treatment, which caused stress and mortality over the short term. Following this experimental trial, fish were observed to feed and grow when held in culture conditions of 3 ‰ (30 g fish) and 40 ‰ (2-100 g fish) for longer periods of time (about 2-3 months).

Maturation
The aim of this trial was to monitor, in on-farm pilot-scale systems, the rate and timing of maturation of 0+ flounder entering their first reproductive season. Previous trials have shown that relatively high rates of maturity were detected in spring in 1+ flounder juveniles. It was the intention of this trial to map the progress of maturation through percentage maturation and to correlate this with growth, temperature and time of year. Unfortunately unrecorded stock movements and grading in the facility made this difficult. The highest incidence of maturation in captivity appears to occur between July and September.

Flounder production
Flounder juveniles were produced as a part of the research and these fish were transferred to growers for trials. Interestingly some of the fish developed spinal deformities following transfer to these grow-out facilities, suggesting that feeds (salmonid) used at the time may be unsuitable for flounder and may have contributed to the deformities. About 5-10,000 juveniles were produced in total.

Meetings
Meetings were held with growers on-site and at the University during the duration of this project. A major meeting was held on 24 June, 1997 and included a workshop on flounder larval rearing and discussions held on the establishment of a disease surveillance program, nutrition, deformities and directions for future research. A list of the participants is included as an Appendix.

KEYWORDS: Greenback flounder, feeding, maturation, osmoregulation, spinal deformities

2. BACKGROUND

Greenback flounder (*Rhombosolea tapirina*) has been considered a potential candidate for culture in cool temperate regions of Australia (Hart et al., 1993; Ritar et al., 1994; Searle and Zacharin, 1994). A summary of the benefits and problems associated with the culture of this species is outlined in the FRDC final report 93/234 - "The culture performance of the greenback flounder (*Rhombosolea tapirina*) under grow-out conditions" (Purser, 1996).

Since 1989 considerable effort has been directed toward the development of hatchery techniques (Hart, 1991,1992, 1994, 1995; Hart and Purser, 1995, 1996; Hart et al., 1996) and grow-out techniques (Purser, 1996; Purser and Thomas, 1995) with research programs being undertaken at the University of Tasmania, Launceston and DPIF Division of Sea Fisheries, Taroona, Tasmania. The ecology of the species in the natural habitat was investigated by Crawford (1984). Further studies have examined feeding and nutrition (Carter et al., 1996; Shelverton, 1996; Bharadwaj, A.S., 1997), stress and handling (Barnett and Pankhurst, 1998), reproduction (Barnett, 1998), sensory development (Pankhurst and Butler, 1996) and population genetics (van den Enden, 1996).

The preferred market size appears to be 300 g+ with a premium for 500g+ fish. Preliminary market assessments for flatfish in Australia and overseas form part of the first FRDC report on flounder. There is a large diversity of flatfish species available in the market place in Asia in particular. It is therefore difficult to assess markets without placing the product into the market place. Recent cultured product trials into Taiwan by a local grower received an offer of $13/kg fresh and approximately $20-30/kg live. The markets require a consistent supply, size and quality which gives the cultured product a premium price over fresh or frozen trawl product, especially when the cultured product is presented live.

The market in Australia is more difficult to assess accurately because there appears to be a regional response toward the product. Northern states are less familiar with flounder or other flatfish while the product is much more acceptable in the market in the southern states. Active market promotion would appear to form an essential and large component of the future production of flounder for the Australian market. Operators would not be able to rely solely on the present market price or perception of the product as is the case with more widely recognised high price species. Flounder is currently perceived as a low priced species but this is based primarily on inconsistent supply and size, and more specifically on frozen imported product.

From the FRDC study (Purser, 1996) a number of issues were identified that required attention. Of these, issues such as osmoregulatory capability in relation to siting of farms and tolerance to freshwater flushes, deformities which affect the marketability of the product, and feeding and maturation which influence the growth of the animals were selected to be part of this grant application. These particular areas of study were selected as preliminary elucidation of some aspects of these problems was achievable in the short time frame available in which to undertake the study: less than 12 months. More comprehensive data could obviously be obtained through longer term studies. The results from this study therefore serve to answer some of the basic questions and to direct future research.

3. NEED

Numerous research projects at the Department of Aquaculture, University of Tasmania over the last 8 years have contributed to a significant database on various aspects of greenback flounder culture; included was the FRDC grant 93/234 on the grow-out. After examining this data, the principal investigator in conjunction with industry representatives, identified a number of key areas which were considered bottlenecks in or impediments to, production and the commercial development of this species.

These included the need to:

- Better understand the mechanism and causes of deformities and mal-pigmentation, as this reduces the marketability of the end product.
- Produce juveniles for grow-out trials in the pilot scale operations established by industry players; no other Tasmanian facility was producing juveniles for this purpose at the start of the grant.
- Determine the feeding patterns and preferential feed intake times, given that flounder could perhaps feed both during the light and dark cycles.
- Identify the response by flounder to changes in the salinity, as some sites were influenced by flooding and freshwater run-off at various times of the year; it would also assist in identifying suitable sites.
- Construct a profile of the maturation of the fish, as this affects the growth and marketability.
- Develop a specifically formulated diet for this species and over a longer term identify ways of reducing the reliance on fish meal and improve the utilisation of carbohydrates. This also should have flow-on effects in fish health and growth.
- Identify methods to inhibit the maturation process, given that reduced growth lengthens the overall time to market size, increasing production costs.
- Compile more comprehensive marketing data on prices, sizes, volumes and quality.

This study examined most of the above with the notable exception of the last three items. The overall aim was to extend the scientific database in relation to these issues and to answer some of the questions raised by growers, which were directly relevant to culture conditions.

4. ORIGINAL OBJECTIVES

(i) Identify the stage(s) during flounder larval/juvenile development when deformities occur.
(ii) Determine the effect of nutritional feed supplementation and environment on the deformities in the juvenile fish.
(iii) Establish a diel feeding profile for juvenile flounder, particularly in relation to light and dark cycles.
(iv) Measure the changes in blood osmolality during exposure to various salinity levels.
(v) Construct a profile of maturation and relate this to water temperature and growth.
(vi) Produce juveniles for grow-out trials (as a consequence of producing fish for experimentation).

5. RESEARCH METHODS, RESULTS AND DISCUSSION

Experiment 1 - Spinal Deformities

Investigation into the timing of spinal deformities in hatchery reared greenback flounder (*Rhombosolea tapirina*)

Craig Thomas and John Purser

Introduction

Deformities and skin mal-pigmentation (albinism, pseudo-albinism) are two conditions observed during the production of greenback flounder. Neither is unique to this species, with similar conditions being reported in other flatfish species, such as halibut and turbot. Numerous papers have investigated the possible causes of mal-pigmentation proposing the influence of light or substrate (Liewes, 1984), low DHA levels in the larval diet which influences stress levels and general condition (Dhert et al., 1994), deficiency of vitamin A, DHA and phospholipids hindering retinal rhodopsin development (Kanazawa, 1993), low DHA:EPA ratios (Reitan et al., 1994; Lavens et al., 1995) and the sub-optimal nutritional content of *Artemia* compared to copepods (Naess et al., 1995). Few studies have examined spinal deformities in cultured flatfish. Dedi et al. (1995) demonstrated that excessive vitamin A induces spinal deformities in Japanese flounder larvae. Koukouvas (1995) recorded a higher level of lordosis and scoliosis in greenback flounder weaned prior to metamorphosis. The principal cause of scoliosis and lordosis in most fish appears to be vitamin/mineral deficiency or imbalance of chemicals such as inositol, ascorbic acid, manganese and phosphorus (NRC, 1993).

Although both albinism and spinal deformity are perceived as problems for marketing, the latter has been considered by growers as the major problem because of its additional effect on growth and development. Interestingly, when fish displaying both conditions were presented at the taste test trials as part of the previous FRDC grant, the mal-pigmented fish were considered the least desirable. Under commercial conditions, fish displaying either condition, could conceivably be filleted but this may return less to the growers compared with live fish. It is desirable therefore to understand the causes of these conditions and minimise the level of incidence.

In past trials high percentages of spinal deformities, particularly around the peduncle, have been detected in juveniles (10g+). A proportion of fish used in growth trials displayed this condition, and it was thought at the time to be possibly caused by a nutritional deficiency.

This short study aims to identify the time during larval and early juvenile development when spinal deformities are visibly detectable in the greenback flounder and to suggest directions for testing in the future to resolve this problem. Although one trial concentrating on the development of deformities was undertaken for this experiment, information from a further two production trials is incorporated into the results and discussion for comparison.

The second objective, to identify the effect of environment and nutritional supplementation on the deformities, was to carry on from the first part of this experiment however it was realised

that this experiment encompassed a huge design matrix and consequently this was not conducted. Therefore data on the effect of feeds and environment are only descriptive at best.

Materials and Methods

Broodfish

One 300g female flounder brood fish was hormone induced (single dose of ovaprim 0.5ml/kg) on day 1 and the eggs removed by manual stripping on day 4. Eggs were fertilised with milt from 2 males using the dry fertilisation method.

Egg Incubation

Following fertilisation, eggs were washed several times in 0.2 um 35 ‰ seawater and the sinking non-viable eggs removed prior to incubation. Eggs were incubated in 1um filtered seawater at 12 ^{o}C in 80 L tanks. The water in the incubating tanks was lightly aerated and an exchange rate of 20-25 % per hour was used through recirculation. A fertilisation rate of 50 % was determined 2-3 hours post-fertilisation at the 4-8 cell developmental stage. Hatching occurred in the egg incubation tanks on day 3 post-fertilisation; larvae were transferred to larval rearing tanks after hatching.

Larviculture

Yolk-sac larval fish were stocked into a 180 L black hemispherical tank on day 4 post-fertilisation at a density of less than 30 fish per litre. This tank was equipped with a 63-250 um outlet screen and serviced with light aeration and a low water exchange rate of 8 mL/sec in a recirculation system. Fish were maintained in this tank until weaning at which point they were transfer to 250 L grey reln tanks 32 days post-hatch (= dph).

Feeding

Algae
Approximately 8-15 L of a the green motile microalgae *Tetraselmis suecica* was added to the culture per day to maintain a cell density of 30, 000 cells per mL in the tank from the time of stocking 1 dph until weaning commenced 32 dph.

Rotifers
Rotifers were enriched in a 50: 50 mix of the algae *Pavlova lutheri* and *Isochrysis* (T. *iso)* after harvesting from culture tubs 24 hours prior to feeding. During the last 12 hours Frippak was also added. The rotifers were fed to fish at densities of 5 per mL twice per day: in the morning and afternoon. Few rotifers remained in the tank prior to the next feeding, as some flushing was used. Rotifers ceased being distributed to the larvae 18 dph.

Artemia
Initially, instar 1 AF grade Artemia were introduced 17 dph for 2 days. Instar 2 AF grade Artemia enriched in Frippak were fed there-after until the completion of weaning 50 dph.

Weaning
Weaning commenced 32 dph and finished 50 dph. During weaning, the Artemia ration was gradually decreased in increments over the 17 day transitional period. Artemia were offered frozen (enriched) rather than live toward the end of the weaning period. A 50:50 mix of 0.6 mm Biodiet and Nippai diet was fed over the weaning period using a combination of hand feeding and automatic belt feeders.

Dry Diets
The initial dry diet 50:50 mix of 0.6mm Biodiet and Nippai was introduced at the commencement of weaning 32 dph and was used until 60 dph. At this time fish were large enough to take a Gibson low oil 1mm crumble. This diet was gradually introduced from 55 dph and was maintained until fish with spinal deformities were first identified 90 dph. The trial was terminated at 94 dph after the visual identification of spinal curvature.

Identification of Spinal Deformities

During the larval and early juvenile stages, fish were sampled and visually assessed for spinal deformities at critical stages of development and/or changes in feed (ie start of exogenous feeding, flexion of notochord, metamorphosis, pre-weaning and post-weaning). Once fish were weaned, close visual inspection of fish was undertaken every 7-10 days for curvature of the spine particularly in the caudal fin area.

Results

Experimental Trial
Development of the flounder from first feeding through to the completion of the trial is outlined in Fig. 1. Positive visual identification of spinal deformities occurred at 90 dph when the fish were about 40 mm in length. No sign of spinal deformity was observed prior to this. Deformities were observed 58 days after the start of weaning and 40 days from the completion of live feeds.

Low levels of head and eye deformities were observed prior to and during weaning, but these related to incomplete metamorphosis.

Production Trials
Although not part of the spinal deformity experiment, two additional batches of flounder juveniles were reared but Selco products were used instead of Frippak/micro-algae during the larval phase. Lansy diet was used instead of Nippai and Biodiet during the weaning and subsequent on-growing following the success found by Cheetham Salt in minimising deformities. I must emphasise that none of these diets has been formulated for flounder but have been used on the basis of availability. The results in this report are no reflection on the overall quality of these diets.

The first production batch displayed very low levels of spinal deformity (< 1 %) in fish of 18-21 mm (0.08-0.14 g) and an age of 63 days post-hatch. Despite the low incidence of spinal deformities, the mal-pigmentation rate was 19-35% within the 6 tanks. This low incidence of spinal deformity did not increase over time in this batch when monitored at 5g average weight. These fish were maintained on a Lansy NRG diet.

The second batch displayed higher levels of spinal deformities at 34 % in fish of 35 mm (1 g) and 126-143 dph. Mal-pigmentation rate in these fish was lower at 13 %. These fish were also being fed Lansy NRG diets though this was complicated by the older age of the diet compared to batch 1 above and the need to feed salmon diets for 1 week due to the unavailability of Lansy diets. The egg quality and survival rates also were poorer in batch 2 compared with batch 1.

Summary of results:

Batch	Age (dph)	Length (mm)	% Deformity	% Mal-pigmentation	Larval enrichment	Juvenile diet
Exp	90	40	not counted	not counted	Frippak/algae	Biodiet/Nippai
Prod1	63	18-21	<1	19-35	Selco	Lansy
Prod2	126-143	35	34	13	Selco	Lansy + trout

In conclusion, spinal deformities were observed visually at a range of sizes and ages in the flounder. Despite this variability, the deformities were visible after the weaning period in all batches and not during the feeding of live feeds.

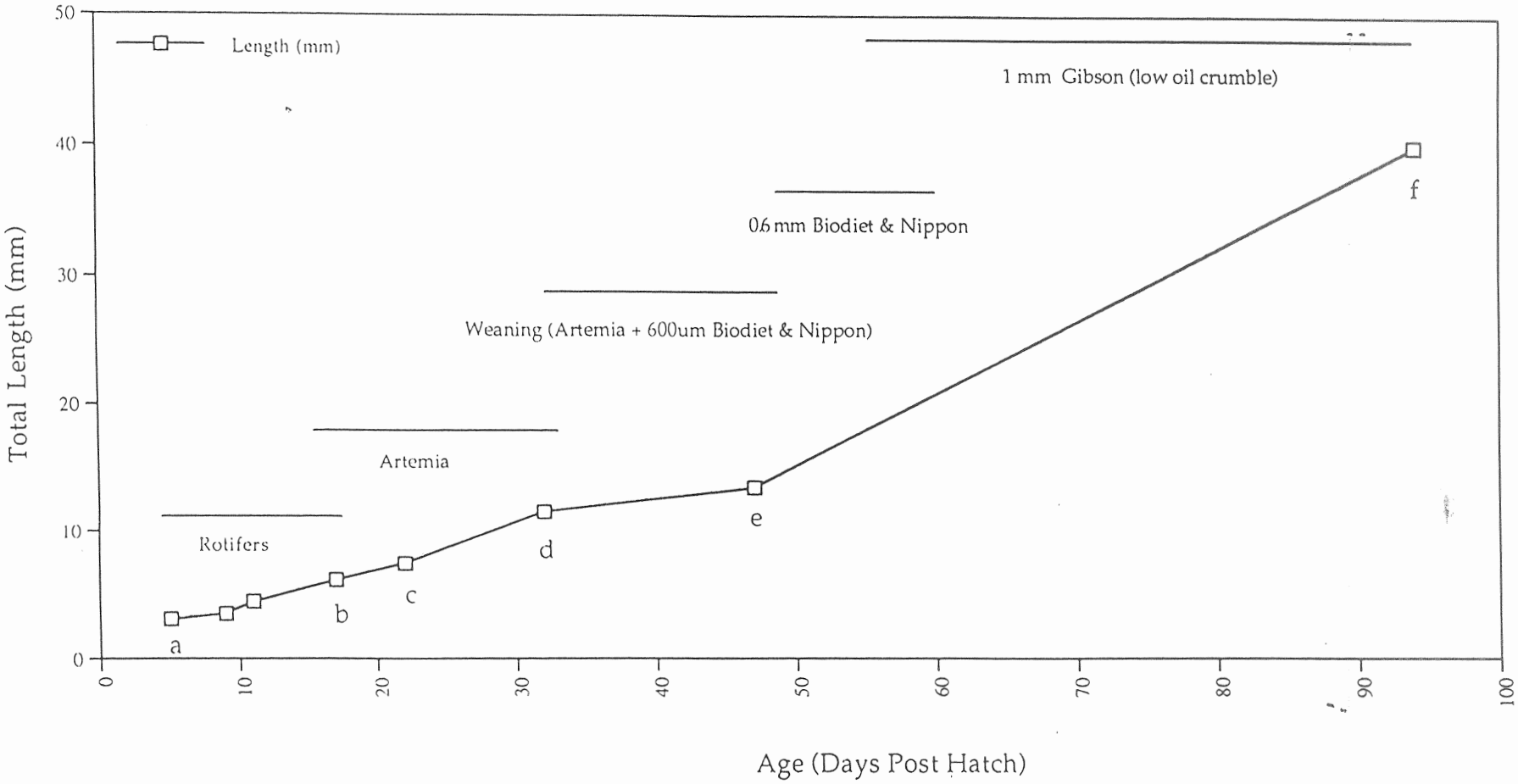


Fig 1. Length, feeding protocol and developement of greenback flounder from hatch until 94 days investigating the timing of spinal deformation in hatchery reared juveniles. [a-commencement of exogenous feeding; b- flexion of notochord; c- metamorphosis; d-Pre-weaning; e-Post-weaning; f- first evidence of spinal deformation day 94.]

Discussion

In the experiment, spinal deformities were observed in juvenile fish averaging 40 mm in size and 90 dph in age. Subsequent production trials showed deformities in fish of 35 mm and 126-143 dph, with few detected in another batch of 21 mm and 63 dph. It appears that the time when the deformity is visibly detectable does vary but is consistent in that it only seems to occur post-weaning, after the fish have been feeding on artificial diets.

Although the deformities were visible at this stage and size, the mechanism(s), which produced this condition in the fish is unknown. There are a number of critical factors and stages between fertilisation and the expression of spinal deformities post-weaning that may cause the condition, or at least aid its expression. These include temperature, enrichment diets, vitamins A and C, fatty acid levels spread over the developmental stages of egg incubation, hatching, first feeding, metamorphosis, weaning and post-weaning. It was this complexity in factors and the necessary experimental design that forced the authors to re-assess the feasibility of undertaking the experiments to identify possible causes of the deformities. The best guess, based on available literature (NRC, 1993), suggests that it is likely to be a nutritional problem, but it could also involve other conditions such as environmental factors, fish condition and growth. There is however no evidence to scientifically support these suggestions at this point in time for this species.

Some interesting comparisons may be made between the present study and that by Koukouvas (1995), in which four different weaning regimes were compared with a live feed control, in terms of growth, survival, condition and health issues, such as scoliosis and lordosis. A ten day weaning period were used starting from day 4 post hatch (regime 1), day 15 (regime 2), day 26 (regime 3) and day 37 (regime 4). Rotifers were provided at 3 dph until 12 dph and Artemia were distributed from day 8 until the end of weaning in all regimes. The feeding regime used in the present study is most similar to regime 4. Spinal deformities were observed at different times through the study and in general 23-29 days after the start of feeding artificial diets and 14-20 days after the completion of live feeds. This compares with 58 and 40 days in this study. Lordosis and scoliosis levels were 60 & 45 % (regime 1), 91 & 63 % (regime 2), 15 & 17 % (regime 3) and 0 & 0 % (regime 4 and control). Deformity levels were higher in regimes started before metamorphosis (1, 2 & 3) than the one started after (regime 4). The current study initiated weaning post-metamorphosis, hence comparison is best made with regime 4. No deformities were recorded in regime 4, however the study was completed at 67 dph which is much sooner than the 90 dph recording of deformities in the present study.

Some juvenile flounder on-grown in pilot scale tank systems have developed spinal deformities after a couple of months growth, possibly suggesting that the feed was inadequate nutritionally, as all environmental factors were within an acceptable range.

Although comparison between trials is not possible because of the differences in protocol and lack of control, the experiment has shown that high incidences of spinal deformity do exist in some batches, and that the condition is visible during grow-out but not earlier than weaning. Although it points to inadequacies in the grow-out diet, other factors such as poor egg quality, poor larval quality, poor live feed nutrition and sub-optimal environmental conditions cannot be ruled out. There are no data to suggest possible causes; at best it is speculative. The major problems in deciphering this issue lie in the fact that no data exist on the nutritional

requirements for this species and the experiment to identify the optimal conditions (or adverse conditions which induce the condition), are complex. It is known though that some batches produced display low levels of spinal deformities, so the conditions in some production runs are suitable to produce healthy juveniles.

Experiment 2 - Feeding profiles

Feeding rhythms in tank-reared greenback flounder (*Rhombosolea tapirina*)

Wei min (Peter) Chen and John Purser

Introduction

In the wild, juvenile greenback flounder feed predominantly on invertebrates such as amphipods, copepods, mysids and polychaetes with their feed intake and activity related to a variety of factors such as prey species, time of day, tidal cycle and age of fish (Crawford, 1984).

The diel feeding pattern identified by Crawford (1984) in her study on the ecology of the species, showed that in newly metamorphosed fish, feeding occurred during the day whilst in older juveniles, feed intake occurred both diurnally and nocturnally. Both vision and olfaction appear to be important to feeding in *R. tapirina*, with olfaction becoming increasingly important with age. This is consistent with the development of the olfactory rosette and lamellae formation sometime after metamorphosis (Appelbaum et al., 1983; Kawamura and Ishida, 1985; Pankhurst and Butler, 1996).

Fish tend to move up and down with the tide (tidal rhythmicity or circatidal activity) but this does not appear to be correlated with feeding. This movement onto sandflats during the high tide during the day and night may be a predator avoidance response as seen in some European flatfish species such as plaice (Gibson, 1973) and English sole (Toole, 1980).

Although details of the feeding behaviour and hierarchies has been reported by Shelverton (1995) and Carter et al. (1996), no data on the preferential feeding times or the diel rhythms has been collected for greenback flounder. This is relevant in optimising feed utilisation, particularly as flounder are non-aggressive feeders, preferring to ingest pellets from the tank floor rather than the water column. They also are a stomachless flatfish, with an inability to quickly ingest large meals.

It is essential to understand the feeding requirements of the fish in a culture environment to optimise feed intake. The commonly used approach allows the fish to determine their preferential feeding time and feed intake by making feed available to them at all times. This approach has been studied in a number of species in recent years and has become possible only through the development of essential technology incorporating electronic sensors and computer programs. Two types of systems are used to measure feeding response: demand feeders or waste pellet monitoring systems. The first records the number of activations of the feeder over time as an indicator of the needs of the fish, while the second measures waste pellets after the fish have had the opportunity to feed on a predetermined (usually in excess of satiation) quantity and set frequency of feeding. Many of the studies overseas use demand feeder technology to continuously monitor feed input (Boujard, 1995; Sanchez-Vazquez et al., 1994). It does not record the actual feed ingested though but does illustrate when feed is wanted. In more recent trials this has been overcome by combining both demand feeders and waste pellet monitoring systems (Madrid et al., 1997). Waste pellet monitoring systems have also been used singly, but most deliver feed at set intervals so that both input and output are

known, and actual consumption calculated. The weakness in this system is that fish are allocated feed in excess but not necessarily at the frequency they choose.

The authors are not aware of any studies in the literature on the detection of feeding profiles of flatfish in tank-based systems using a pellet monitoring system, though some data are available in a study on demand feeders (Burel et al., 1997).

In visual feeders such as large juvenile salmonids, feed is delivered only during the light phase to optimise growth and FCR, and minimise waste. However, in visual and olfactory feeders such as the flounder, can growth be maximised by feeding 24 hours per day? In the wild, where environmental conditions change and feed is available at certain times, the older juveniles tend to feed more during the day but do also feed at night to some extent. The question is: under culture conditions where feed is not restricted and environmental conditions are held constant, do the flounder show the same patterns as the wild fish or do they display a different preferential feeding time. If they prefer to feed all day, growth may be improved on the present figures by extending feed availability.

This study aims to utilise an Aquasmart waste pellet monitoring system to establish a diel profile of preferential feeding in both individual fish and small groups of this flounder species.

Materials and methods

1. Fish culture conditions

Flounder for experiments were maintained in a seawater recirculation system, comprising a culture tank, waste pellet monitoring system, solids removal, biofilter, reservoir and pump. The specifications are outlined in the following description.

A central draining plastic circular tank with a volume of 300 litres and a surface area of about 1 m^2 incorporating a single tangential water inlet, which produced clockwise spiral currents to the central outlet, was employed to hold the fish. The floor of each tank had a slope of 3^0 from the outer edge to the central outlet. The flow rate was measured by a rotameter installed in the inlet pipe. The waste pellet sensor was placed in the outlet pipe 100 cm below the central outlet of the tank. The waste pellet monitoring system is described in more detail in the following section.

The experimental system was placed in a temperature-controlled room and the tank was enclosed by a black plastic sheet to minimise any disturbance to the feeding behaviour in the fish. Illumination was provided by a fluorescent tube mounted over the tank, giving an intensity of 70 lux at the surface of the water. The light was controlled by a timer that turned the light on and off without a twilight period. A photoperiod of 12L:12D (dawn at 08:00 and dusk at 20:00) was used. Other environmental factors measured during the course of the experiment were: flow rate = 9 l/min, water temperature = 14°C, salinity = 30-32 ‰, dissolved oxygen >7.5 mg/L, and pH = 8.1.

Greenback flounder ranging in size from 95 g to 195 g were used in the experiment. These fish were randomly collected from a cultured population maintained in a 2000L tank under an artificial illumination with the photoperiod mimicking the ambient cycle. Following transfer into the experimental tanks, a period of 5 to 14 days was provided to the fish to acclimatise

them to the new environment prior to the trials. Individual fish and small groups of flounder were used in the system.

During both the acclimatisation and experimental period fish were fed on 4 mm pelleted salmon diet (Gibson's Ltd, Tasmania) with an average weight of 49 mg/pellet and composition of 42% protein, 22% lipid, 9% ash and 6.5% moisture.

During the trials, pellets were distributed to the fish by a mechanical belt feeder, described below.

2. Waste pellet monitoring system

The integral components of the waste pelleted feed monitoring system (hereafter referred to as the monitoring system) were an electronic sensor and a data recording unit both connected by a water-proof cable. The sensor was located in the outlet of the tank and detected the number of passing waste pellets, which in turn was recorded by the data recording unit.

The sensor was calibrated for each type of pellet under specific environmental conditions (eg. clearness of water and flow rate) to allow recognition of the pellet type and to distinguish it from faeces that could pass through the sensor. The monitoring system was capable of recording data continuously over time. Cumulative numbers of the uneaten pellets were recorded at selected time intervals. The data in the system was transferred into a portable computer via a 9-pin serial port for later analysis. The components of this system were designed and manufactured by Aquasmart Pty Ltd and have been used mainly on salmonid seacage farms to date.

A mechanical belt feeder was used to deliver pelleted feed to the fish. The belt was marked into 30 sections through a calibration; each section taking 30 minutes to deliver its pellets. The number of pellets on each section and the start time for activation of the belt were recorded manually. The number of pellets in each section corresponded to a feed level slightly higher than satiation, as determined prior to the experiment. The monitoring system was set to start recording at the same time as the feeder started moving. Thus, the number of waste pellets recorded by the monitoring system could be converted into the feed intake with reference to the time of day. Feed intake, within every half an hour, was calculated by the following formula:

Feed intake (number of pellets/half an hour) = pellets presented (number of pellets/half an hour) – waste pellets (number of pellets/half an hour)

The feed intake was then converted into weight units by multiplying this feed intake by the average weight of a pellet (mg/pellet). Relative feed intake (mg-feed/g-fish) in half an hour was used to illustrate the feeding patterns in the fish.

Experiment 1

Initially, the feed intake of 5 individual flounder was measured to identify if there was a significant difference between fish in terms of intake and pattern of intake. This was performed on individual fish to remove the effect of hierarchies and competition from other fish, as found in a previous study. Each trial lasted for three consecutive days. A 2 day

starvation period was used prior to monitoring to nullify prior feeding patterns. Ten pellets (0.49 g) were dispensed to individual fish every half an hour by a mechanical belt feeder as described above. This number of pellets were determined to be in excess of the satiation level for any given 30 minute block of time. Waste pellets were monitored for three days and data expressed as a quantity of feed intake per 30 minute block of time.

Experiment 2

The feed intake of a small group of three flounder was assessed to compare with the intake of the individual fish in the previous experiment. Three of the fish in experiment 1 were used for this trial. Thirty pellets (1.47 g), which were determined to be in excess of the satiation level, were distributed to the group every 30 minutes using the belt feeder as described above. Waste pellets were monitored for three days and the data expressed as a quantity of feed intake per 30 minute block of time.

Weight and length of the fish were measured at the end of the trials instead of the beginning to minimise stress on the fish.

Experiment 3

The trial was repeated with 5 fish but undertaken over an 8 day period after the starvation period was found to influence the intake pattern. Pellets were delivered in excess to the fish every 30 minutes and the pellet intake calculated per 30 minute block of time.

Data presentation

In a similar fashion to many feed ingestion trials in the literature, data are presented graphically as actograms to illustrate the general pattern of feeding preferences. Actograms consist of relative feed intake expressed on the y-axis, time intervals in a day on the x-axis and consecutive days displayed vertically. Statistical analysis is not essential at this level as the aim of the trial was to describe the diel pattern of feed intake, not to determine periodicity which is undertaken in circadian rhythm studies.

Results

Experiment 1

The relative food intake per half an hour throughout three consecutive days in each of five individuals is presented as actograms in Figure 1(A)-(E). It was found that the fish fed frequently and ceased feeding occasionally. Each fish showed small fluctuations in the daily food intake, but differences in the daily food intake between individuals was considerable. For example, fish 1 ingested 7.31 g daily which was 7.5% of the body weight; in contrast, fish 2 only took 5.22 g daily which equated to 2.68% of the body weight (Table 1).

Data from this experiment suggest that flounder feed both during the light and dark phases almost at an equal level (Figure 3).

Experiment 2

The feeding pattern of the group of three fish (fish 3, 4 and 5, Figure 2) was similar to that of individuals (Figure 1), indicating no preferential feed intake period. However, daily food intake of fish held in the group exhibited less variation than those held individually. The average daily food intake of the group of three fish (13.83 g) was less than the sum of the average daily food intake of three individuals held in isolation (15.95 g) (Table 1).

The feeding pattern expressed by the diel actograms of the individuals and the group, suggest that there is no obvious feeding rhythmicity displayed over this short period of time following a bout of feed deprivation. The mean feed intake for the individual fish and group for a 12 hour scotophase and a 12 hour photophase is shown together in Figure 3. In general fish both individually and as a group ingest similar amounts during both light and dark phases. In the group, slightly more is consumed during the photophase.

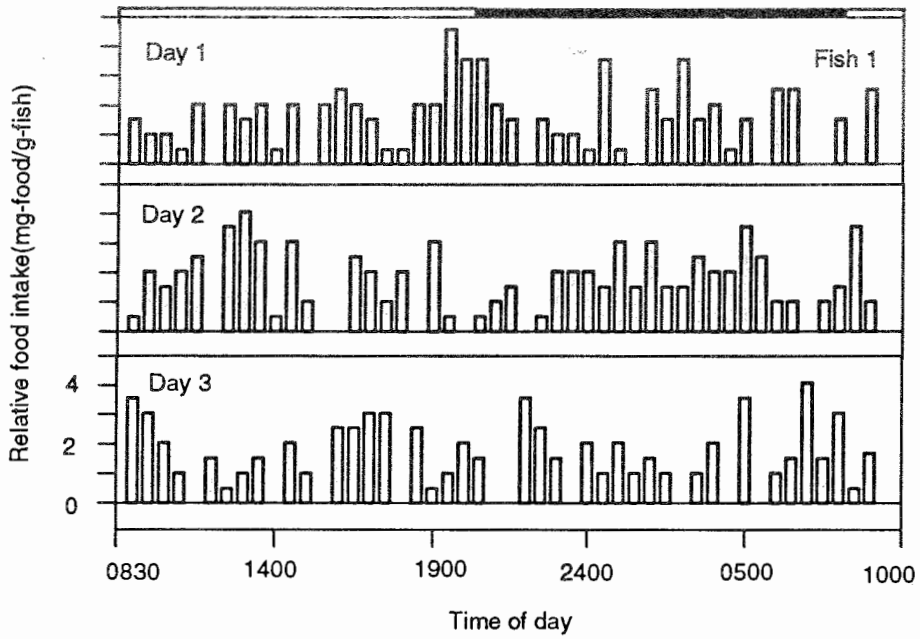
Experiment 3

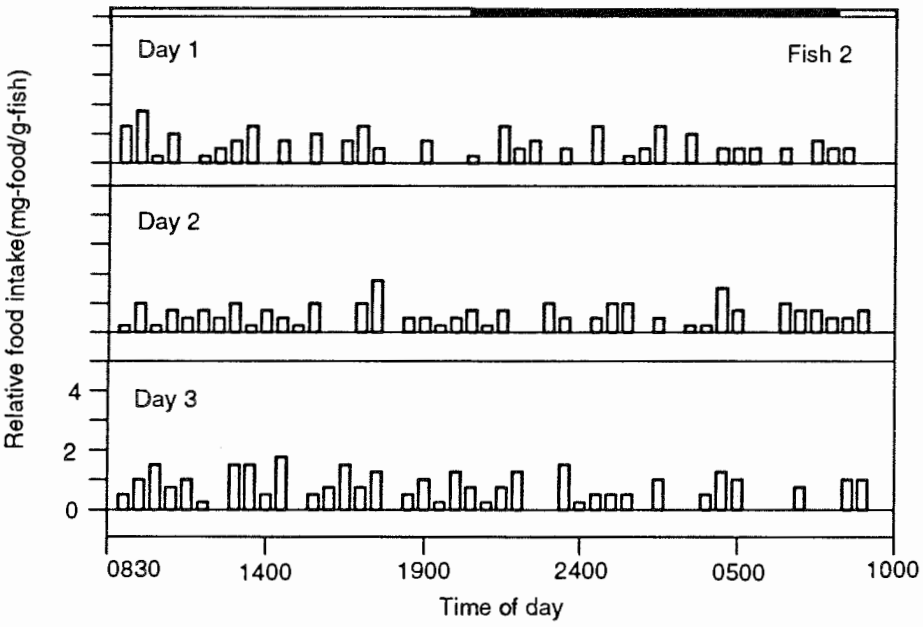
This experiment was conducted to follow on from the previous two experiments after a pattern change (not shown) was observed in a longer term preliminary trial. The pattern found in this experiment clearly shows a diurnal feeding pattern with feed intake predominantly occurring during the photophase (Figure 4). It was found that little or no rhythmicity was detected over the first 1-2 days supporting the results from the first two experiments. After acclimation to the feed delivery and satisfaction of the previous hunger from the feed deprivation period, the fish displayed a settled, preferential feeding rhythm. Therefore, the results from experiments 1 and 2 are useful in that they show the feeding behaviour of the fish after a bout of feed deprivation, but do not give a true picture of the preferential feeding times in this species under settled culture conditions.

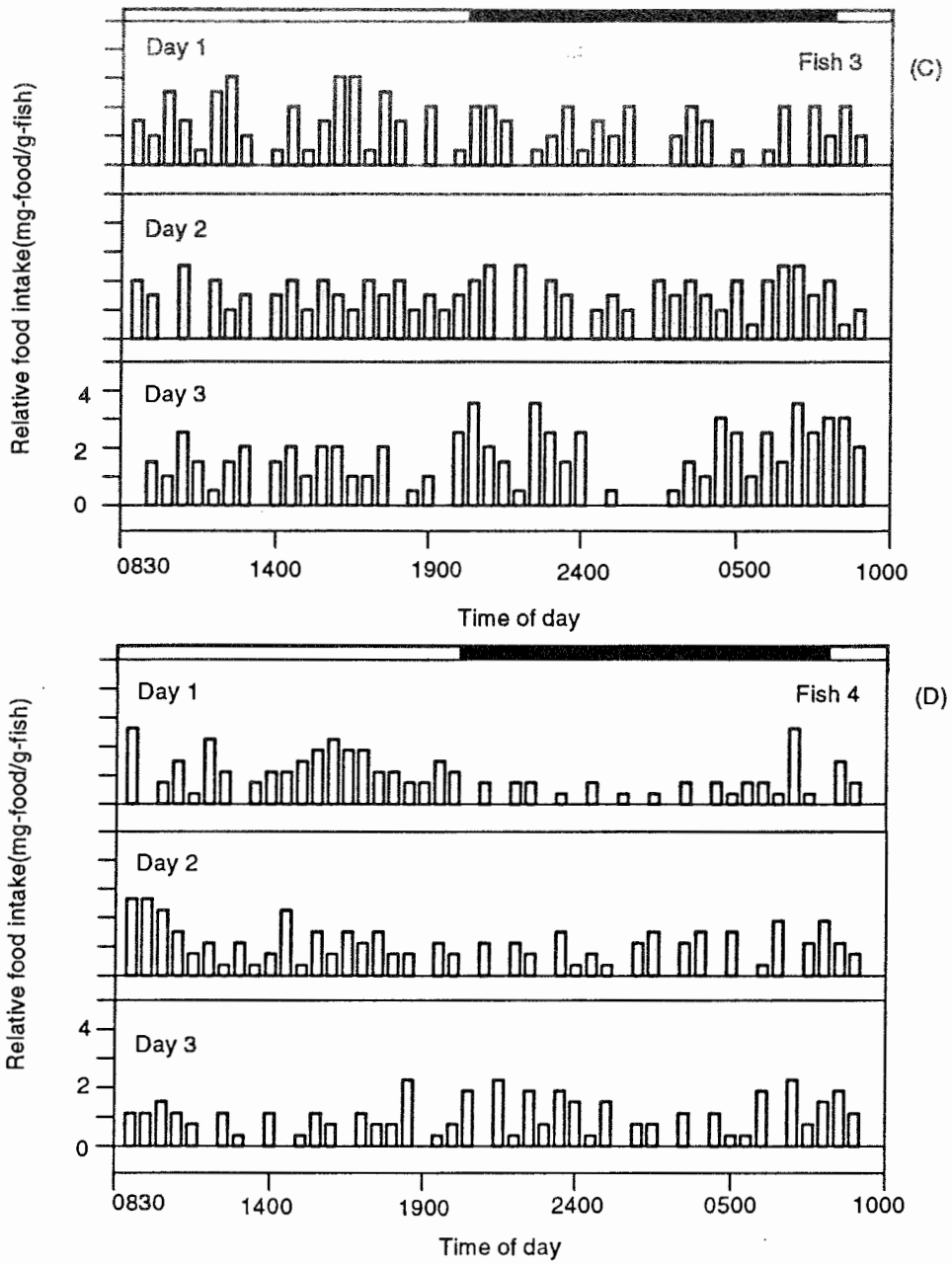
TABLE 1 Daily food intake of five individuals and a group of three fishes

Code of fish	Date of trial	Total length (mm)	Live weight (g)	Daily Ration (g)	Daily food intake (g)				Mean daily food utilisation rate(%)**	% of biomass***
					Day 1	Day 2	Day 3	Mean		
Fish 1	2-5/2/97	190	97.44	23.52	7.15	7.70	7.07	7.31	31.08	7.50
Fish 2	9-12/2/97	225	194.78	23.52	4.58	5.02	6.05	5.22	22.19	2.68
Fish 3	12-15/2/97	195	95.48	23.52	5.67	6.45	7.03	6.38	27.13	6.68
Fish 4	17-20/2/97	212	129.80	23.52	5.26	5.98	5.59	5.61	23.85	4.32
Fish 5	20-23/2/97	194	115.00	23.52	4.66	3.04	4.21	3.97	16.88	3.45
A group*	1-4/3/97		361.70	70.56	16.32	14.99	10.19	13.83	19.60	3.80

*: The group of three fishes was comprised of Fish 3, 4 and 5.
**: Mean daily food utilisation rate(%)= 100% x mean daily food intake/daily ration
***: % of biomass = 100% x mean daily food intake/live weight of the fish;


Day 1
Fish 1
(A)
Day 2
Day 3
4
2
0
Relative food intake(mg-food/g-fish)
0830
1400
1900
2400
0500
1000
Time of day


Day 1
Fish 2
(B)
Day 2
Day 3
4
2
0
Relative food intake(mg-food/g-fish)
0830
1400
1900
2400
0500
1000
Time of day


Day 1
Fish 3
(C)
Day 2
Day 3
Relative food intake(mg-food/g-fish)
4
2
0
0830
1400
1900
2400
0500
1000
Time of day
Day 1
Fish 4
(D)
Day 2
Day 3
Relative food intake(mg-food/g-fish)
4
2
0
0830
1400
1900
2400
0500
1000
Time of day

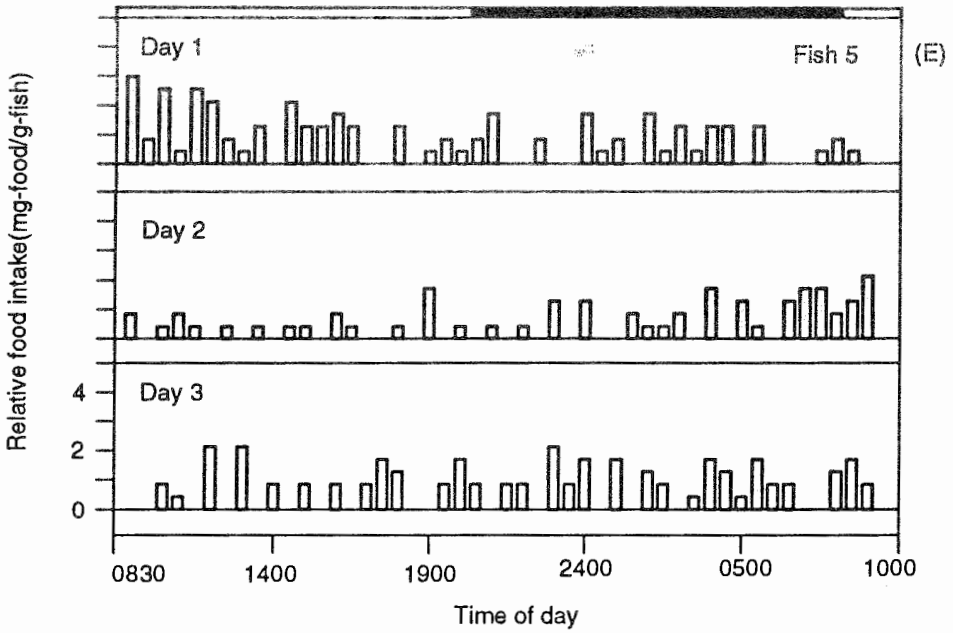


Fig. 1(A)-(E). Feeding patterns of five individuals over three consecutive days. A 12L:12D photoperiod (dawn at 8:00; dusk at 20:00) and 70 lux light intensity was used. Open horizontal bars indicate the light (photo-) phase while the dark horizontal bars indicate the dark (scoto-) phase. Feed consumption is represented at 30 minute intervals.

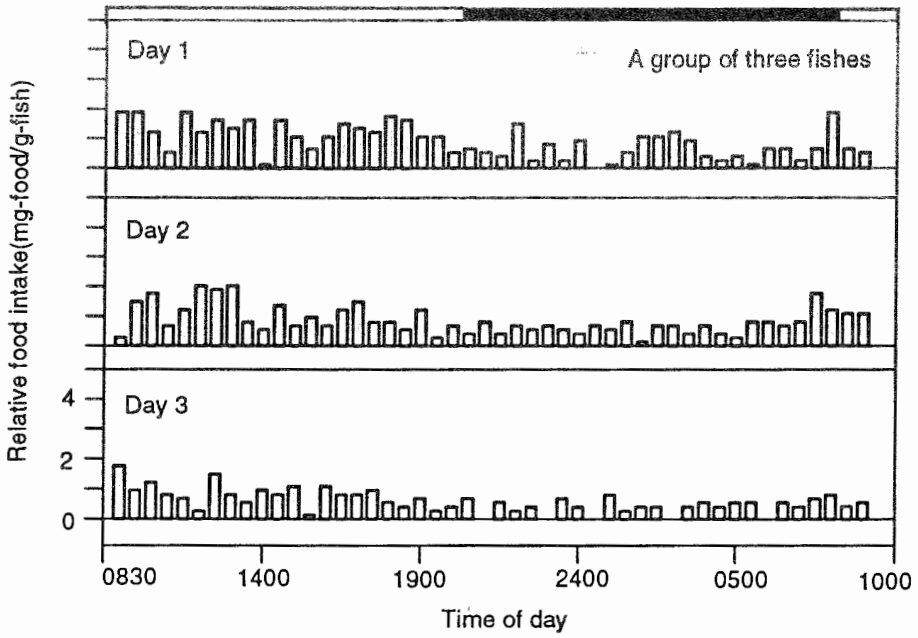


Fig. 2. Feeding patterns of the group of three fishes over three consecutive days. A 12L:12D photoperiod (dawn at 8:00; dusk at 20:00) and 70 lux light intensity was used. Open horizontal bars indicate the light (photo-) phase while the dark horizontal bars indicate the dark (scoto-) phase. Feed consumption is represented at 30 minute intervals.

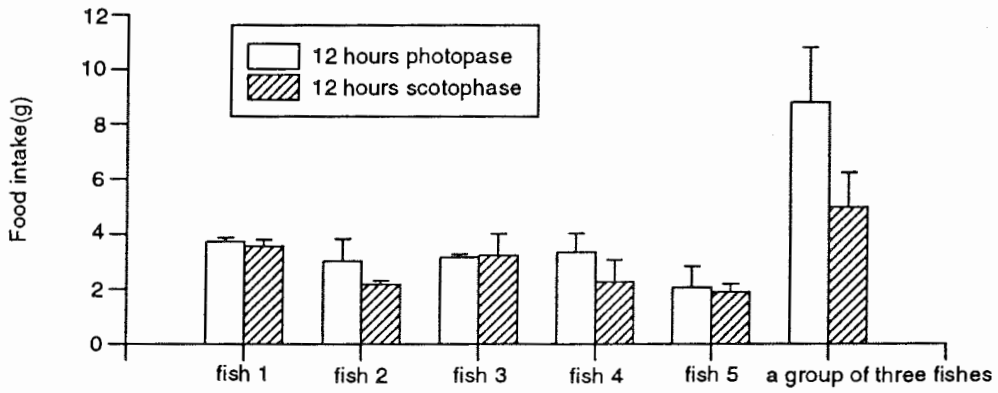


Fig. 3. Comparison of mean food intake between 12 hours photophase and 12 hours scotophase in five individuals and a group of three fishes for three consecutive days, vertical bar indicates standard deviation. Data suggest that feed intake during the light and dark phases is similar in individual fish. Direct comparisons between fish are not valid due to the difference in body weight.

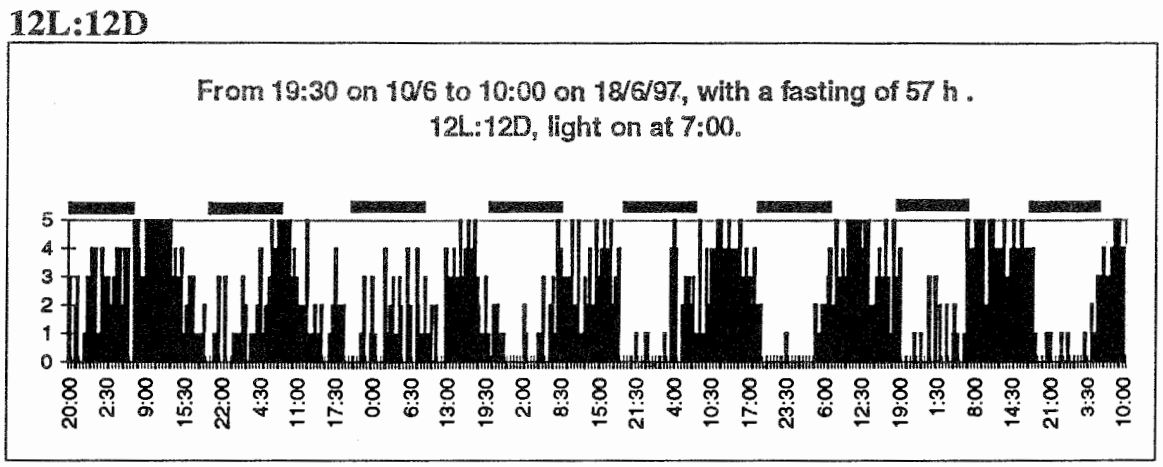


Figure 4: Feeding profile of 5 flounder juveniles in experiment 3 over a period of about 8 days. The graph displays the extended feed intake over the first 2 days following a feed deprivation period of 57 hours prior to the start of the experiment. From day 4, a diurnal rhythm is established where fish showed a preference to feed during the photophase. The dark bars denote the scotophase (dark phase). The x-axis represents time of day over almost 8 days and the y-axis represents number of pellets ingested by the group of flounder, over each 30 minute interval.

Discussion

Under the rearing conditions used in these trials, and with access to feed over each 24 hour period, greenback flounder display a preference to feed during the photophase. Some pellets are consumed during the scotophase, particularly following a starvation period, indicating that this species is quite capable of feeding during the dark phase. This information agrees with the feeding data on wild flounder collected by Crawford (1984).

The starvation period was used in the trials to override previous feeding regimes during the acclimation period. The problem with the first two experiments was that the trial was not conducted over an adequate period of time to correctly identify the normal feeding preferences. This was substantiated over the first 2 days in experiment 3. These experiments, however, did display the preferred feed intake pattern following a bout of feed deprivation. Under these modified conditions, fish fed almost continuously throughout the day (light and dark), possibly through a compensatory feed intake mechanism. Compensatory growth following restricted feed rations has been observed previously in juvenile greenback flounder (Purser, 1996). Under these circumstances, light cues appear to play a secondary role to hunger in determining the feeding times during the day.

The food intake of both individuals and a group of three fishes could be seen as maximum one under the given conditions, because fish were given excess food each 30 minutes; the mean daily food utilisation rate of the pellets delivered ranged from 16.88 to 31.08%.

In experiments 1 & 2, the food intake of greenback flounder was similar during both the 12 hours photophase and scotophase, indicating that senses such as olfaction are probably as important as vision in detecting food. There is variation in the daily feed intake of individuals and group with the group of fish consuming less than the combined intake of individual flounder. This may be an artifact as the trials were conducted on separate days, even though the same individual fish were used. It may also be possible that hierarchical influences within the group are affecting individual intake (Carter et al. 1996). The share of the meal consumed by individual fish within a group was not determined in this trial.

There is variation between the intake of each of the individuals, with some fish consuming considerably more than others. This pattern is not unusual, but what is interesting is the high percentage intake observed in some of the fish. A couple of the fish ingested 6-8% body weight/day which is much higher than the 2-3% of the others; the latter being close to the anticipated satiation level. It is possible that this high level of ingestion also suggests that compensatory feeding occurs following feed deprivation.

The results of feeding rhythms in greenback flounder were obtained at a light intensity of 70 lux and photoperiod of 12L:12D. More work in the future is required to examine the feeding rhythms under different light intensities and photoperiods, and at ambient light conditions, which would be experienced under culture conditions.

Experiment 3 - Osmoregulation

Effect on flounder of direct transfer to water of different salinities

Peter Girling, John Purser and Barbara Nowak

Introduction

Most studies in the literature concentrate on the transfer of fish from freshwater to seawater. Many of these involve salmonids, with very few studies having investigated the response of marine species transferred from salt- to fresh-water.

The growth rates and feed conversion efficiency of the aquaculture flatfish species, turbot (*Scophthalmus maximus*) and summer flounder (*Paralichthys dentatus*) have been shown to be better at salinities of 15-25 ‰ (Scherrer, 1984; Gaumet et al., 1995) and 20 ‰ (Malloy and Targett, 1991; Daniels et al., 1996), respectively, than in seawater.

Little information is available in the literature about the effects of salinity on greenback flounder. Crawford (1984) found juveniles and adults distributed through a wide range of coastal salinities suggesting that the species is euryhaline in nature. However, apart from data on the effects of salinity on the eggs and larvae of this species (Hart and Purser, 1995), no data are available in the literature on the tolerance of flounder juveniles to changing salinities, particularly rapid changes. This area is of importance to flounder culture having relevance to site selection, stock performance, health and feeding. Before the performance of the fish exposed to different salinities can be assessed, it is essential to establish the tolerance and response of this species to changing salinities.

This experiment aims to describe the osmoregulatory response of this species to direct salinity transfer by measuring plasma osmolalities.

Materials and Methods

Feed deprived (2 days) flounder were transferred directly from the holding facility (33‰, 945 mOsm) to 100 L Nally™ bins filled with 60 L of water adjusted to the desired salinity. Five fish were randomly distributed to each bin, with a different bin of flounder being used at each blood sampling time. Fish were only sampled once. Total number of fish used = No. salinities x No. sampling times x 5.

Salinities tested were 40 ‰, 15‰, 7‰, 3‰, fresh water (FW i.e. 0‰) and a control (i.e. transfer to 33‰.) These salinities were created using an appropriate mixture of sea water and aged tap water. Tap water was aged by vigorous aeration for at least 24 hours, and was found to be soft (total hardness 20 mg/L, comprised primarily of calcium at 12 mg/L) with pH= 6.5. Raised salinity i.e. 40‰ was achieved by the addition of laboratory grade NaCl to sea water. Tank water was aerated by a single airstone, static and unfiltered. Water exchange was not necessary in these trials, as total ammonia and pH levels were not found to alter significantly in this time. Total ammonia remained below 0.2 ppm during all trials. pH ranges, as a consequence of the salinity, can be found in Table 1. Temperature remained constant at 16.5°C.

Table 1: pH ranges for the duration of each salinity transfer trial.

Trial	Minimum pH	Maximum pH
Control	8.2	8.3
Transfer to 40‰	8.2	8.4
Transfer to 15‰	7.3	7.6
Transfer to 7‰	7.2	7.4
Transfer to 3‰	6.9	7.3
Transfer to FW	6.2	6.5

Five fish (one tank) were anaesthetised using 50 ppm benzocaine and blood sampled at one sample time over the 48 hour sampling period (as shown in Figure 1.). A different tank was used at each time. Time zero (T_0) samples were taken from the fish still in sea water. Blood was extracted by caudal puncture using a heparinised syringe and needle. Blood was placed in Eppendorf tubes and centrifuged at 6,000 rpm for 3 minutes. The supernatant (plasma) was removed and analysed for osmolality using an Advanced™ micro-osmometer.

Statistical analyses were conducted using JMP v3.2.1 (SAS Institute INC.) statistical package. ANCOVA was used to compare plasma osmolality changes over time and ANOVA was used for comparisons between results taken at a single time point. All data were tested for normality using the Shapiro-Wilk W test on residuals, and for homogeneity of variance using Cochran's test. A significance level of $P<0.05$ was considered significant in all tests.

Results

Survival

No mortalities were recorded following transfer to salinities greater than fresh water. In contrast to other trials tested here, transfer to fresh water elicited a severe response in the fish and was stopped 16 hours post-transfer. This response was characterised by appearance of mucus on the water surface of the tanks four hours post-transfer, and by 12 hours post-transfer the fish were unresponsive in a semi-anaesthetised state with a visibly depressed and irregular ventilation rate. At 16 hours there were two mortalities (6% of the fish remaining in fresh water) and the trial was aborted with the remaining fish transferred to 15‰ for recovery.

Recovery was slow, with some improvement in ventilation rate and responsiveness after four to six hours. Further mortalities were recorded from the recovery tanks, 43% of those fish exposed to 16 hours in fresh water did not successfully re-acclimate to sea water following an eight-hour phase in 15‰.

Plasma osmolalities

Plasma osmolalities of fish were significantly affected after transfer from sea water to salinities ranging from fresh water to 15‰ (see Figure 1). Statistical analysis using ANCOVA found the whole model to be significant, with both hours post-transfer and salinity treatment to significantly contribute to the fit of the model (ANCOVA; both $P<0.001$).

In addition to the whole model, a comparison of every treatment with the control was also considered. No significant difference was detected between treatments at T_0, (ANOVA; P=0.872), whereas osmolalities of fish from each treatment, except transfer to 40‰, were found to differ significantly from the control across the entire sampling period (ANCOVA; P<0.001).

Responses of the plasma osmolalities to the range of salinity transfers conducted can be classified into three types (Fig. 3.1). Response type one occurred after transfer to 40‰, and was unique in its slight tendency to increase whilst not deviating significantly from the control for the entire trial.

The second response type was elicited by transfer to both 15‰, 7‰ and 3‰. Although they differ significantly from the control, these treatments were not found to be significantly different from each other (ANCOVA; P=0.185). This response is characterised by an initial decrease in osmolality during the first four to six hours, followed by a plateau for the remainder of the trial. The gradients of these treatments were not found to be significantly different from the control from six hours post-transfer onwards (ANCOVA; P=0.152).

The final response type followed transfer to fresh water. Osmolality dropped continuously for 10 hours post-transfer, with a short plateau from four to six hours. This decrease was followed by a slight but non-significant increase from 10 to 14 hours before falling again. Initially the osmolality did not fall faster than other treatments, with no significant difference detected between the values at two hours post-transfer for either the 15‰, 7‰, 3‰ or FW treatments (ANOVA; P=0.284).

Discussion

Plasma osmolality of the greenback flounder after transfer to increased salinity i.e. 40‰ tended to rise, although not significantly. Rises in plasma osmolality have long been documented following transfer to increased salinity (Kirsch and Mayer-Gostan, 1973; Bath and Eddy, 1979), and were probably not detected in this transfer due to the relatively small salinity increment tested.

Numerous phases have been described in fish following salinity challenge. Houston (1959) was the first to describe these phases, defining the crisis phase as a period of rapid and significant change, leading up to the stabilisation phase in which homeostatic mechanisms regulated internal conditions to a stable value within the tolerance range. Since this study, these phases have been described in a number of species, eg tilapia (Bath and Eddy, 1979), barramundi (Almendras, 1996), and European eel (Kirsch and Mayer-Gostan, 1973).

After transfer from seawater to either 15‰, 7‰ or 3‰, plasma osmolality dropped significantly for four hours before remaining steady for the remainder of the trial. This four-hour crisis phase compares favourably with studies on eels, tilapia and barramundi which found ionic turnaround times of two hours (Kirsch and Mayer-Gostan, 1973), three hours (Hwang et al., 1989), and four hours (Almendras, 1996) respectively.

Direct transfer to fresh water was found to be too extreme for the osmoregulatory ability of this species. The continuous decline in plasma osmolality was briefly interrupted by a short and unsuccessful attempt at regulation from four to six hours post-transfer consistent with the standard turnaround time found in other salinity transfers. This attempt at regulation was not continued, with osmolality continuing to decline until 10 hours post-

transfer. From 10 hours post-transfer osmolality stabilised, before a final decrease until mortalities began at 16 hours post-transfer.

The pH values vary according to the salinity, as the freshwater used was quite soft. These were not manipulated artificially because these were the resultant values after mixing the fresh- and sea-water available. In future trials, it may be useful to determine if the mortalities found in the freshwater treatment were directly from osmoregulatory stress, from a low pH or a combination of the two factors. Exposing the fish to buffered freshwater would resolve this issue.

In conclusion, this section has found the greenback flounder to be extremely tolerant of salinity transfer over the short term. The duration of the crisis phase following salinity challenge is comparable with other major euryhaline species, which coupled with rapid re-stabilisation of internal conditions suggests this species to be highly adapted for a rapidly altering environment.

Culture conditions experienced by this group and others (Peter Rankin, pers. comm) suggests that flounder are capable of growing in low (5 ‰) and high (40 ‰) salinities for longer periods (in terms of months).

The next step would be to test the tolerance of the fish over a longer period of time, simultaneously recording the feed intake, osmolality and growth to identify if the growth potential at a range of salinities is comparable to that in seawater. If so, a broader range of sites would then be available for flounder culture. There may be other benefits; other factors such as diseases may be controlled by varying salinities for longer periods of time than would normally be used, or a lower incidence of disease may be experienced at lower salinities.

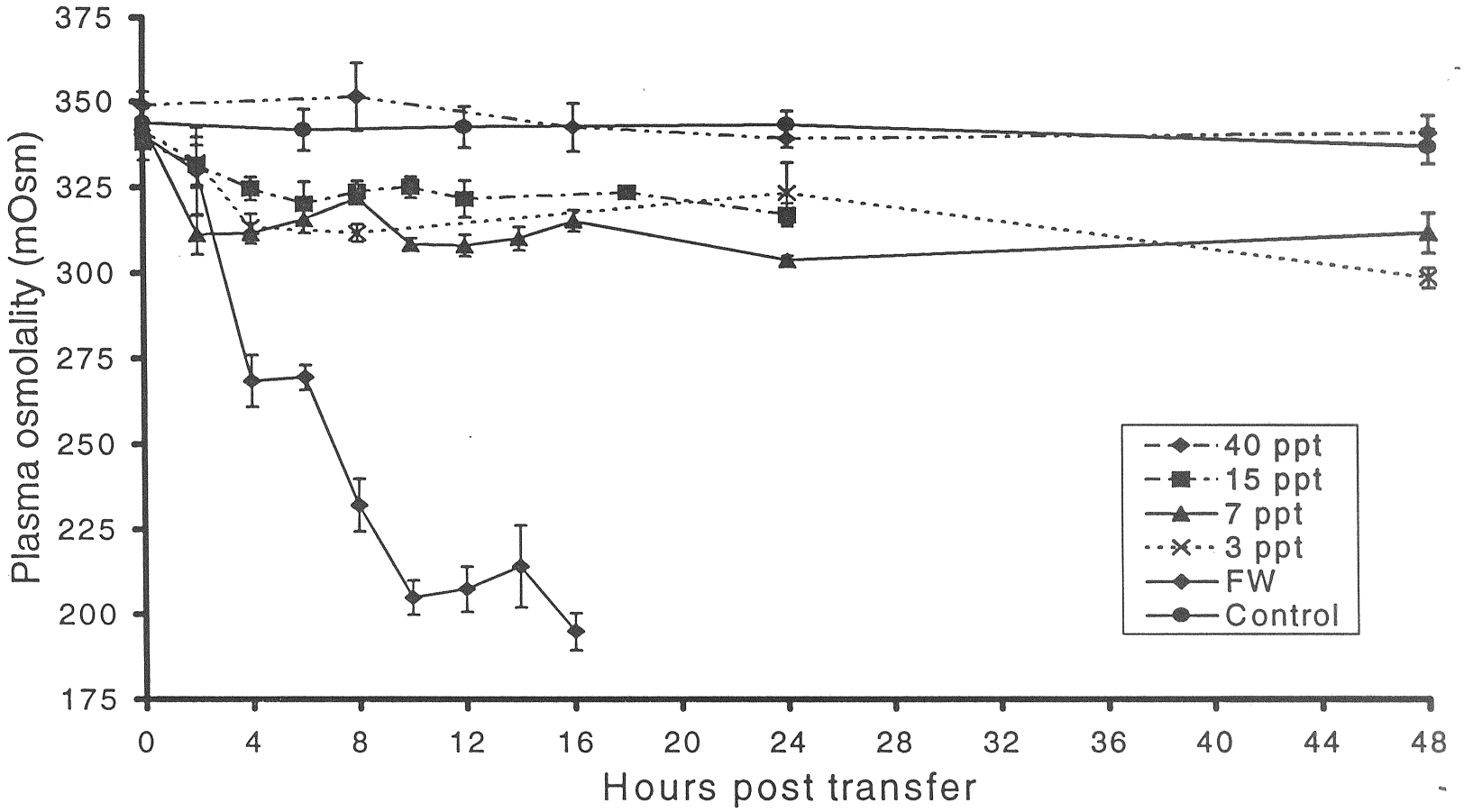


Fig. 1: Plasma osmolalities (mean ± S.E.; n = 5) following direct transfer from 33‰ to various salinities.

Experiment 4 - Maturation

Maturation in greenback flounder

John Purser and Craig Thomas

A trial was set up to establish the degree of maturation which occurs in the fish over a period of time leading up to and including the spawning period between April and October. Testing of correlations between growth, size, sex and degree of maturation was to be undertaken on selected batches of juvenile flounder located on a pilot-scale grow-out site.

Although the trial was started, it was only conducted over a couple of months because the fish were accidentally sorted and mixed on the farm discontinuing any consistent monitoring strategy.

Results from the grow-out trial (Purser, 1996) showed that a relatively high maturation rate existed at the time of sampling in August: 83.6 % fish were mature (51.8% male and 31.8% female), at an average size of 166 mm and 106 g. In this trial the figures that were available showed the levels of maturing females to be: in April 2% of small fish (139 mm and 56 g) and 10% of the larger fish (198 mm and 164 g) and in early June, 26 % of the graded population (population average = 188 mm and 139 g).

From the trials undertaken over time and through observation, the main period of spawning and ovulation appears to be August and September under ambient conditions. Under temperature and photoperiod control, broodstock have been brought into spawning condition as early as March and continue as late as December/January but the quality of eggs in these fish is relatively low compared with the fish which spawn in July to October. Ripe males are not always available during this earlier time period either. The spawning can be prolonged by maintaining the temperature below 13°C and the photoperiod on about 8:16 L:D (short days).

It has also been found during the grow-out trials that fish as small as 20 g and 1 year old begin to mature.

Although it is convenient for continuous hatchery production to have ovulating females and ripe males year-round, it has a negative impact on the growth of the grow-out fish. Feeding is not optimal as a consequence of maturation and, in addition, energy is diverted to gonadal rather than somatic growth. Work on reproductive endocrinology and spawning is continuing at the University of Tasmania under the supervision of Prof. Ned Pankhurst. This work is addressing a number of issues regarding captive broodstock and handling. This work is vital in the development of broodstock management techniques for the production of good quality eggs and larvae on a year-round basis.

Another area of importance is the development of techniques to impair the maturation of juveniles during the grow-out phase so that growth is not compromised. A number of techniques are suggested including triploidy, environmental manipulation and restricted feeding practices. These areas should form the focus for any future research work in this field.

6. BENEFITS

The potential benefits are:

- Significant levels of deformities have been experienced in the juvenile fish post-weaning. These may cause growth problems and certainly would affect market perception, price and acceptability. Once the time frame when deformities occur and the type of feed involved have been identified, the nutritional composition of the diet can be examined or the diet changed to overcome the deficiency or imbalance. The benefits are in the improved growth and body definition for market, and the removal for the need to grade/cull deformed fish from hatchery stocks. It would also contribute partly to the understanding of the nutritional requirements of the species.

- Understanding the feeding behaviour and preferences of this species would potentially improve the growth, FCR, condition factor and reduce the variability in size. The benefit here involves improved growth and performance, which reduce grow-out time to market and optimises feed utilisation improving overall profitability.

- Although it has been presumed that the best sites are full strength seawater sites, it is possible, based on their distribution in the wild, that estuarine sites are also suitable. It is unclear what effect floods may have on the saline sites. Before growth under these conditions is examined, it is important to determine the physiological response of the fish to these conditions. Osmolality of the fish is an indicator of the ability of the fish to adapt to these changing conditions. Hypersaline conditions in salt farms and in inland water ways (e.g. ground water) may also prove suitable for this species extending its range of potential sites even further. The benefit here relates to the flexibility in siting farms and understanding the osmoregulatory response of the animals to fluctuations in salinity.

- Maturation in the fish inhibits growth and affects survival. Early maturation is a particular problem because the majority of fish have not reached market size. Before methods to inhibit the maturation process can be developed, it is essential to identify the general profile of maturation in these fish. Ultimate benefits created by inhibiting early maturation include shorter production time to market, more uniform growth, improved feed utilisation and reduced disease incidence (maturing fish have a depressed immune system).

- Potential beneficiaries of the study as outlined in the application are growers or potential growers, investors and researchers. The states which may benefit and relative proportions are Tasmania (70%), Victoria (20%) and South Australia (10%).

7. FURTHER DEVELOPMENT

The project has provided information on most of the original objectives set. Additional information would benefit these fields but useful and useable baseline data has been compiled. The main areas that proved difficult to comprehensively study in the time frame set were: profiling maturation over a period of time and determining the effect of feed ingredients and environment on the degree of deformities.

I feel the main areas, which require urgent attention in the study of this species are:

- detailed studies into the causes of spinal deformities and mal-pigmentation in flounder,
- identification of the nutritional requirements of this species,
- formulation of a suitable feed and more specifically a cost-effective diet, and
- prevention of early maturation in the fish which inhibits growth (and time to market) and affects product quality.

As an extension of these topics, additional study areas are:

- market assessment using cultured quality fish, and
- effect of formulated diets on flesh quality and colouration.

8. CONCLUSIONS

Deformities

- The objective to identify the timing of the appearance of the deformities through the larval/juvenile rearing process, was met. The objective on the effects of feed supplementation and environment was met only superficially.
- Spinal deformities were detected in the juvenile fish post-weaning; none were visually identified pre-weaning.
- It suggests that the weaning diet, process of weaning or grow-out diet may contribute to the deformities or at least contribute to the expression of the deformities.
- The use of the Lansy diet during weaning rather than a commercial Japanese weaning diet or salmonid diet greatly reduced the incidence of spinal deformity in the fish in one trial. Fish at an average weight of 5 g and 8 months of age have expressed very low levels (<1%) of spinal irregularities in recent trials. However, this result is based on separate batches of larvae rather than experimentation on the same cohort. This needs to be tested in a scientifically designed experiment to confirm (or otherwise) this observation.
- As much as could be measured, the environmental conditions remained unchanged to those used in previous trials in which deformities were identified.
- Poor egg and larval quality leading to decreased growth and survival, despite using the same feeding protocols, may also contribute to deformities. This is speculative at the moment and should be checked.

Feeding

- The objective to identify the diel feeding profile in flounder juveniles was met.
- Flounder juveniles are capable of feeding during light and dark cycles but appear to display a preference to feed during the light cycle.
- Juveniles will consume comparative quantities during both the dark and light phases if a bout of feed deprivation has occurred; when continuous satiative feeding is used fish prefer to feed during the photophase.
- Individual fish display a similar pattern of feed ingestion, but this level of intake varies considerably between individual fish following a short period of feed deprivation.

Osmoregulation

- The objective to measure the changes in blood osmolality during exposure to various salinity levels after direct transfer, was met.
- Flounder juveniles are euryhaline in nature, being able to adapt after direct transfer to a range of salinities over at least short periods of time. Fish displayed a slight reduction in the blood osmolality following direct transfer from seawater (33‰) to salinities down to 3‰ over a 48 hour period and a slight increase in salinities up to 40‰.
- Transfer to freshwater resulted in mortalities and a high stress level after 6 hours. About 40 % of the fish returned from freshwater to seawater via acclimation subsequently died.

Maturation

- The objective to construct a profile of maturation and relate this to water temperature and growth was started but was unsuccessful due to accidental mixing of fish on the farm. Some general aspects and observations on maturation are however discussed.
- August to September appear to be the major times of the year when maturation levels in the fish are high. Degrees of maturation have been detected in fish from about March. Fish as small as 20 g can show increases in gonadal weight.

- Flounder respond to changes in the environment with the on-set of maturation controlled by light and temperature manipulation. Fish held under constant light and temperature regimes tend to mature later than those reared under ambient conditions.
- About 2% of small fish and 10% of larger fish were maturing females in April, while 26% of fish were maturing females in June.

Juvenile production

- The objective to produce juveniles for on-growing, as a consequence of the experimentation, was met. Fish were transported to 2 farms.

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10. APPENDICES

Appendix 1

ATTENDANCE LIST - MEETING

List of participants at the meeting held at the University of Tasmania, 24 June 1997.

Growers/potential growers

Peter Rankin, Cheetham Salt, Lara, Victoria
Bill Swan, Swan's Greenback Flounder, Port Huon, Tasmania
Alec Purves, Purves Fisheries, Bridport, Tasmania
David Krushka, Smithton, Tasmania

Tasmanian DPIF

Dr Piers Hart, Sea Fisheries, Taroona
Dr Judith Handlinger, Fish Health Laboratories, Launceston
Dr Jeremy Carson, Fish Health Laboratories, Launceston
James Watson, Fish Health Laboratories, Launceston
Dr Paul Hardy-Smith, Field Veterinarian, Hobart

University of Tasmania

Dr John Purser, Department of Aquaculture, Launceston
Dr Chris Carter, Department of Aquaculture, Launceston
Mr Craig Thomas, Department of Aquaculture, Launceston
Dr Barry Munday, Department of Aquaculture, Launceston
Mr Tony van den Enden, Department of Zoology, Hobart

Invitees who could not attend -

Mr Malcolm Lovell, Tasmanian Development and Resources, Hobart
Mr Peter Chew, Pipe Clay Marine Farms, Clifton, Tasmania
Mr David Wright, Pipe Clay Marine Farms, Clifton, Tasmania
Mr Scott Wright, Pipe Clay Marine Farms, Clifton, Tasmania

Appendix 2

STAFF

Funded by FRDC -
Mr Craig Thomas

Contributed to the program-
Dr John Purser
Dr Barbara Nowak
Mr Peter Girling
Mr Peter Chen
Mr Peter Chew
Mr David Wright
Mr Scott Wright

Appendix 3

INTELLECTUAL PROPERTY AND VALUABLE INFORMATION

The trials outlined in this report have been kindly funded by FRDC and supported by in kind contributions from the Department (now School) of Aquaculture, University of Tasmania (all trials), and Pipe Clay Marine Farms (maturation trial). Two of the experiments (feeding patterns and osmoregulation) were incorporated into much larger projects run as a Master of Science and Honours programs, respectively. The author gratefully acknowledges the support of FRDC in these particular components and the project in general. All relevant staff engaged on research experiments in this project have been acknowledged as authors in the relevant sections.