THE EFFECT OF DIET TYPE AND FEEDING RATE ON GROWTH, MORPHOLOGICAL DEVELOPMENT AND BEHAVIOUR OF LARVAL AND JUVENILE GOLDFISH *Carassius auratus* (L.).

THESIS

Submitted in fulfilment of the requirements

for the Degree of

MASTER OF SCIENCE

RHODES UNIVERSITY
GRAHAMSTOWN

by

TIMOTHY GUY PAULET

February 2003

To my parents, Tim and Gil, who made it all possible, thank you.

THE EFFECT OF DIET TYPE AND FEEDING RATE ON GROWTH, MORPHOLOGICAL DEVELOPMENT AND BEHAVIOUR OF LARVAL AND JUVENILE GOLDFISH *Carassius auratus* (L.).

I. Table of Contents

II. Acknowledgements

First and foremost I would like to thank my supervisor, Dr. Horst Kaiser, for his advice and patience throughout the progress of this thesis. His willingness to provide assistance at all hours is greatly appreciated. It would not have been possible without your input and time – thank you! Thanks go to The Liberty Life Education Fund for providing the funding that made this project possible.

Thanks also go to Terry Longman for building the micrometer device that enabled the accurate measurement of mouth-gape as well as for providing technical advice in all practical areas.

Special thanks go to Olaf Weyl for critically reading through chapters of this thesis at a time when I most needed help and to his wife Michelle for proof reading certain sections of earlier drafts. I would also like to thank Cindy for proof reading the General introduction chapter of this thesis- your input was most helpful!

A final thank you goes to Robyn for supporting and motivating me throughout this thesis.

III. Abstract

Intensive grow-out of goldfish, *Carassius auratus* (L.), larvae and juveniles in closed re-circulating systems requires the control of environmental conditions and feeding. This study investigated the effect of different diets, environmental condition and feeding level on growth, development and survival of goldfish larvae and juveniles. Prey preference for *Artemia* nauplii or decapsulated *Artemia* cysts as well as agonistic behaviour was recorded.

The micrometer device used to measure mouth-gape was shown to produce accurate measurements which could be used to estimate the maximal particle size that can be ingested by goldfish larvae of a particular age.

Goldfish completed metamorphosis earlier with an increased feeding level of *Artemia* cysts and by making the cysts more accessible to the fish using up-welling water movement. Morphometric plasticity in goldfish larvae was exhibited within two weeks of growth and it may be possible to induce changes in morphology by manipulating diet and rearing environment. Fish that fed on moving prey items had a significantly larger mouth-gape than those that browsed cysts from the tank bottom or dry food items. The development of mouth-gape was not affected by the feeding level of cysts. Condition factor increased with an increase in the number of cysts fed per fish per day.

Goldfish larvae and juveniles grew faster and had a higher survival when fed on decapsulated *Artemia* cysts than on instar I *Artemia* nauplii or a mixed live/dry diet of *Artemia* nauplii and dry food. Feeding at least 155 cysts per fish per day, in tanks with up-welling water movement, gave the best growth and survival and the smallest size variation. In addition, cysts remained available to the fish for longer periods, and were easier to

prepare and feed. Goldfish larvae preferred decapsulated *Artemia* cysts to nauplii and rejected fewer prey items as they grew older. The frequency of agonistic behaviour increased as fish grew but no cannibalism was recorded for cyst-fed fish.

This study showed that decapsulated *Artemia* cysts are a good alternative to *Artemia* nauplii as a diet for larval goldfish. Good growth and high survival was achieved for cyst-fed goldfish larvae and juveniles at 23 ± 1.5°C and at an initial stocking density of 12 fish per litre. This research also contributes to an understanding of feeding behaviour and attempts to minimise under- or over-feeding of *Artemia* cysts in order to reduce grow-out costs due to the high value of the feed type.

Chapter 1

General introduction

The ornamental fish industry

Presently more than 300 species of fish are reared world-wide and this number is increasing continually with the development of rearing techniques and improved feed quality (Watanabe (1985) cited in Abi-Ayad & Kestemont, 1994). Improved techniques for marine food-fish larviculture since the early 1980's have greatly enhanced the growth and survival of freshwater ornamental fish larvae (Dhert *et al.,*1997), largely through improved technology regarding live food culture and larval rearing practices. Research developments in larviculture and early rearing technology have allowed 90% of currently marketed freshwater ornamental fish to be cultured (Tlusty, 2002). In the United States, ornamental fish production has the fourth largest value, behind that of catfish, trout and salmon (Tlusty, 2002). The export value of ornamental fish reached approximately US$207 million in 1996 (Bartley, 1999). In 2000, Singaporean farmers produced 123 million ornamental fish worth US$19.6 million (Lim, 2001).

The ornamental fish trade in the US was worth approximately US$1 billion in 1997, of which freshwater ornamentals accounted for 96% of the trade (Chapman & Fitz-Coy, 1997). Goldfish *Carassius auratus* was the third most popular imported fish species into the United States in 1997 (Chapman & Fitz-Coy, 1997). Goldfish are in demand by hobbyists and as baitfish, and, along with the golden shiner *Notemigonus crysoleucas* and the fathead minnow *Pimephales promelas*, they are amongst the three most valuable species of baitfish in the United States (Stone *et al*., 1995; Lochmann & Phillips, 1996).

Goldfish biology and life history

Goldfish originally ranged from China to Eastern Europe where they were bred to achieve a wide range of colours, shapes, and sizes (Scott & Crossman, 1973). Goldfish are now widely distributed world-wide through escapes and introductions. Spawning occurs in shallow, weedy coves during spring and summer in the wild, but goldfish broodstock can be kept in spawning condition independent of season through the manipulation of temperature and photoperiod (Kaiser *et al.*, 2003). Goldfish larvae require specific physical, chemical and biological conditions to survive and grow (Kestemont, 1995). The larvae hatch after two days of incubation at 30°C and measure about 5 mm in total length; after absorption of the yolk sac they begin to feed on zooplankton (Moyle, 1976).

Fish larvae are the smallest self-supporting vertebrates and in order to increase their chances of survival, they need to complete their morpho-functional systems in order to escape predation and to obtain food (Osse *et al*., 1997). The transition from endogenous to exogenous feeding is one of the most critical stages in freshwater fish culture and the availability of suitable food of a suitable size is essential for a high rearing success on a commercial scale (Appelbaum & Uland 1979; Gulbrandsen, 1993; Jähnichen & Kohlmann, 1999). Intensive larval rearing of non-salmonid fish such as goldfish and carp has relied largely on the culture of living food organisms despite considerable effort to provide suitable alternatives (Bryant & Matty, 1980). Among the live diets used in fish larviculture, nauplii of the brine shrimp *Artemia* constitute the most widely used food item. More than 2000 metric tons of dry *Artemia* cysts are sold globally each year to fish producers and hobbyists (Lavens & Sorgeloos, 1996). The demand for *Artemia* cysts has exceeded the supply and prices have risen exponentially, creating a bottleneck for the expansion of hatcheries (Lavens & Sorgeloos, 1996) and increased problems for developing countries in

terms of affordability. Thus, research leading to the use of an alternative diet or at least optimisation of cyst usage can reduce production costs.

In intensive culture systems, under controlled conditions, larval performance at the initiation of feeding is mainly affected by husbandry practices (Sharma & Chakrabarti, 1999). Goldfish larvae require a relatively constant temperature and a continuous food supply. Thus, intensive grow-out is best done in closed systems under controlled conditions using adequate diets (Kaiser *et al.*, 2003). Temperature has an important effect on feeding rate and hence growth of fish larvae (Verreth & Den Bieman, 1987) since growth rate increases with an increase in temperature until an optimal temperature for the culture of the species is attained. Kestemont (1995) reported that the highest specific growth rate for goldfish larvae was obtained with a water temperature of 28°C. Photoperiod and light intensity are both important for larval fish growth because some species of fish larvae, such as the gilthead sea bream *Sparus aurata* do not feed at night (Tandler & Mason, 1983). Thus, extending the photoperiod may give fish more time to feed and consequently improve their growth and survival.

Mouth-gape and larval feeding

Predator mouth-gape sets the upper limit for potential prey items; this has been shown in several species of cyprinid fish larvae such as grass carp *Ctenopharyngodon idella*, silver carp *Hypophthalmichthys molitrix*, bighead carp *Aristichthys nobilis* (Dabrowski & Bardega, 1984) and, red snapper *Lutjanus argentimaculatus* (Doi *et al.*, 1997). Researchers, however, do not state whether mouth size was measured dorso-ventrally or across the width. Measurement of gape width can be used to estimate the efficiency with which fish ingest prey of different sizes (Arts & Evans, 1987; Cunha & Planas, 1999). For example, Shirota

(1970) recorded differences in the ratio of mouth gape to total length between various species and suggested that fish of several species grew better with increasing mouth-gape to length ratios. The research reported here uses the ratio of mouth-gape to total length to investigate the feeding efficiency of goldfish larvae offered different types of food.

The traditional method of measuring fish mouth-gape involves simply spreading the jaws with forceps and measuring gape with either an ocular micrometer (Hartman, 1958; Wankowski, 1979) or vernier callipers. However, this has limitations (Arts & Evans, 1987). For example, it is difficult to hold the fish steady and the researcher may inconsistently judge the normal open-mouth position. Since dorso-ventrally measured mouth-gape is generally larger than the lateral dimension in deep bodied fish, it is better to measure the smallest dimension of the mouth using a tapered cone (Arts & Evans, 1987). The gape micrometer used by Arts and Evans (1987) measured the smallest internal dimension of the larval mouth and in doing so, the authors assumed that this dimension represented the largest ingestible particle size. The conventional method of measuring mouth-gape using callipers or an ocular eye-piece overestimates the gape of small larvae and underestimates the gape of larger larvae, suggesting that the gape micrometer provides greater sensitivity (Arts & Evans, 1987). In this work, mouth-gape is measured in hundredths of a millimetre to estimate the maximal particle size that can be ingested by goldfish larvae and juveniles at any time during the first fourteen days after hatching.

When larval fish begin to feed, they have a strictly defined threshold of food size acceptance. In the loach (*Cobitidae* sp.) size of first food item was found to be 0.2-0.4 times the mouth size (Ito & Suzuki (1977) cited in Dabrowski & Bardega, 1984). Cunha and Planas (1999) reported a similar relationship for turbot larvae, *Scophthalmus maximus*

L., which had an optimal prey size of 36% of the mouth height and 40% of mouth width. Cunha and Planas (1999) determined that mouth height was a more useful estimate of optimal prey size since variation was low. This assertion differs from one previously described where the authors proposed that the smallest measurement of mouth-gape was a better predictor of the largest prey item that can be consumed. This study, however, utilises a modified version of the gape micrometer device described by Arts and Evans (1987) which should give accurate results.

The relatively small mouth of carp larvae acts as a constraint regarding the size of food items that they can ingest. Small planktonic food, such as protozoa and rotifers, may offer the best growth and survival in these fish (Jhingran & Pullin (1985) cited in Chakrabarti & Jana, 1991). Dabrowski and Bardega (1984) and Schael *et al.*(1991) state that carp larvae, *Cyprinus carpio*, can potentially open their mouths more than the preferred prey size, which may increase the incidence of cannibalism among them in confined conditions.

The relationship between mouth-gape and prey size can be exploited to optimise both ingestion and growth rates under rearing conditions (Cunha & Planas, 1999). However, fish larvae may also select particular prey groups within the range they can ingest, and gape size may or may not be a good predictor of prey selection by larval fish (Schael *et al.*, 1991). This study reports behavioural observations wherein feeding behaviour and prey selection are examined and subsequently related to data obtained in feeding trials.

Larval morphology and developmental plasticity

Larval feeding requirements are different to those of juvenile and adult fish due to the lack of functional structures such as the digestive tract at early development (Fiogbé &

Kestemont, 1995). Goldfish for example, do not have stomachs. Thus, it is necessary to determine the suitability of a particular diet by comparing it with *Artemia* nauplii, a commonly used diet. It is important to know the average age and length at the time of transformation from larva to juvenile because of the marked changes in behaviour, physiology and ecology which accompany metamorphosis (Fukuhara, 1991). For example, larval size in red sea bream *Pagrus major* is more suitable for estimating the onset of metamorphosis than larval age (Fukuhara, 1991) and this has been demonstrated for other fish species. Diet type has also been shown to affect the time to metamorphosis. In goldfish larvae fed *Artemia*, artificial pellets, or an artificial fluid diet, only larvae fed *Artemia* had completed metamorphosis eleven days after hatching, while fish fed other diets were significantly smaller than those fed *Artemia* (Mills *et al.*, 1996). Generally, as larval fish grow, they become increasingly better at feeding (Miller *et al.*, 1992). Gill and Hart (1996) attributed this change in feeding success to an increase in mouth-gape and a greater stomach capacity.

Phenotypic plasticity is the occurrence of alternative forms of morphology, behaviour or physiological state in response to different environmental conditions (West-Eberhard, 1989). In aquaculture, polymorphisms in fish are thought to be phenotypically plastic responses to their rearing environment (Meyer, 1987; 1990) such as type of available prey (Hegrenes, 2001). An example of polymorphism in natural fish populations is described by Nagelkerke (1997) who recorded phenotypic plasticity among *Barbus* species in Lake Tana where 14 different morphotypes of one species were identified. Nagelkerke (1997) hypothesised that the polymorphism resulted from different feeding strategies and diet types. Hegrenes (2001) induced morphological changes in the head size and mouth-gape of the orange-spotted sunfish, *Lepomis humilis*, by feeding them *Artemia* nauplii, mosquito

larvae or meal worms, indicating that it may be possible for aquaculturists to manipulate certain morphological characteristics such as mouth-gape size through diet selection. Osse *et al.* (1997) hypothesised that the differences in relative sizes of body parts and organs between newly hatched fish larvae and post-metamorphic juveniles are due to growth priorities necessarily set during early larval growth. For larval fish to increase their chance of survival, it may be necessary that they first complete the development of functional body parts that will assist in escaping predation and obtaining food. Morphometric development is compared under different feeding and environmental conditions in this study which may contribute to the understanding of larval goldfish growth.

Selecting diets for larval fish

Selection of the optimal prey size to be fed to larvae during early development is very important for the culturing of many fish species and the availability of adequate prey will determine growth and survival (Cunha & Planas, 1999). For example, feeding food items that were too small retarded the growth of carp larvae, *C. carpio* (Dabrowski & Bardega, 1984). One cause of larval mortality is the failure to initiate sufficient feeding before the yolk sac is exhausted (Blaxter, 1986). At low feeding levels, larval mortality increased among larval herring *Clupea harengus* (Munk & Kiørboe, 1986). In this study, an experiment was undertaken to optimise feeding rate of larval goldfish at the start of exogenous feeding in order to reduce mortality and maximise growth. Larvae in hatcheries are deprived of their natural food sources and are therefore subject to potential nutritional deprivations (Minkoff & Villani, 1990).

In order to replace the live food component in larval diets, it is necessary to find a diet that is water stable and can be accepted, ingested, digested and assimilated at rates comparable

to live feeds (Jones *et al.*, 1993). This study compares decapsulated *Artemia* cysts with a mixed diet comprised of a commercially available dry feed mixed with *Artemia* nauplii and a diet of live *Artemia* nauplii alone. The diet that results in the best growth rate, highest survival, lowest size variation or a combination of these factors for goldfish larvae and juveniles is investigated further. A range of feeding rates are tested for the diet type that performed best in the initial experiments and the results will have practical applications for larval goldfish rearing. The effect of feeding too little has been previously documented for many species of fish such as larval striped bass *Morone saxatilis* (Eldridge *et al.*, 1981) and larval herring *C. harengus* (Werner & Blaxter, 1980) where growth was suppressed and duration of the larval stage was prolonged. Taylor and Freeberg (1984) also showed that slow growth due to inadequate feed supply increased the duration of the larval stage and resulted in extended exposure to agonistic behaviour from larger siblings. Under-fed carp larvae had anatomical defects and impaired growth as well as higher rates of sibling cannibalism (Bryant & Matty, 1981).

The use of *Artemia* nauplii in larviculture

Artemia nauplii are used as a standard larval diet in rearing many marine food-fish species and their application to freshwater ornamental fish larviculture has been demonstrated (Bryant & Matty 1980; Dhert *et al.*, 1997). Seven hundred metric tons of dry *Artemia* cysts are sold each year globally for use in larviculture (Sorgeloos *et al.*, 1991). A drawback in feeding *Artemia* nauplii to freshwater fish is that they survive for up to one hour in freshwater and hence fish need to be fed more frequently in freshwater aquaculture (Merchie, 1996). Also, due to the high cost of *Artemia* cysts alternative diets with a comparable nutritional value are needed.

The larval gut is relatively simple at the time of first feeding, with the stomach not fully functioning in many fish species and absent in some species such as goldfish (Holt, 1991). Mills *et al.* (1996) proposed that because goldfish larvae rely heavily on the presence of proteolytic enzymes in their food for digestion, *Artemia* nauplii supply these enzymes while artificially processed diets do not. Furthermore, fish zymogens are activated by invertebrate enzymes (Dabrowski, 1991; Abi-Ayad & Kestemont, 1994; Mischke & Morris, 1998)

Larval fish must be fed instar I nauplii rather than the starved, less viable instar II meta-nauplii, which are transparent, 50% longer, and faster swimming than the instar I nauplii (Dhert *et al.*, 1997). Instar I *Artemia* nauplii consume their own energy reserves, and at water temperatures of 25 to 28°C they develop into instar II nauplii within 6 to 8 hours. Instar I nauplii can be refrigerated before use in order to reduce their activity levels and thus preserve their nutritional quality. This research contributes to an understanding of feeding behaviour and optimal feeding rates for goldfish larvae with applications to minimise under- or over-feeding of *Artemia* cysts or nauplii and so reduce grow-out costs due to the high value of the feed type.

The use of decapsulated *Artemia* cysts in freshwater larviculture

The use of decapsulated *Artemia* cysts is more limited in larviculture than the use of live *Artemia* (Van Stappen, 1996) but has proven to be a good alternative to *Artemia* nauplii for the larvae of several freshwater fish, including tench *Tinca tinca*, grass carp *C. idella*, common carp *C. carpio* (Jähnichen & Kohlmann, 1999), ide *Leuciscus idus*, wels *Silurus glanis*, and barbel *Barbus barbus* (Vanhaecke *et al.*, 1990). Some marine fish larvae such as the Atlantic halibut *Hippoglossus hippoglossus* have also been found to take *Artemia* cysts in their diets (Gulbrandsen, 1993).

In a trial undertaken at the Ornamental Fish Section of the Primary Production Department of Singapore, growth and survival of guppies *Poecilia reticulata* was significantly better among fish fed decapsulated cysts than fish fed a diet of *Moina* (Dhert *et al.*, 1997). Survival rates between groups of larval *C. idella* and *C. carpio* fed either decapsulated cysts or *Artemia* nauplii did not differ significantly (Vanhaecke *et al.*, 1990; Jähnichen & Kohlmann, 1999). No comparison of these diets has been done for goldfish larvae.

The use of decapsulated *Artemia* cysts in both marine and freshwater larviculture has several advantages over *Artemia* nauplii. Decapsulated cysts (1) are more nutritious; (2) can be fed directly off the shelf without the need for a hatchery; (3) can be stored for years if dehydrated and refrigerated; (4) can be dried to keep their buoyancy in the water column; (5) can to be ingested by smaller fish due to their smaller diameter (nauplii and cysts measure 470-550 μm and 200-250 μm, respectively) (Verreth *et al.*, 1987); (6) last longer in freshwater than nauplii and do not leach; and (7) their dry weight and energy content is 30 to 40% higher than that of freshly hatched nauplii (Vanhaecke *et al*., 1983; Vanhaecke *et al.*, 1990; Dhert *et al*., 1997). Because the nutritional value of cysts is greater than that of nauplii there is the potential to use less cysts than nauplii to achieve the same weight gain. The use of decapsulated cysts also advances the possible commercialisation of less expensive cyst products with low hatch rates, since even improper harvesting and processing of cysts does not affect their nutritional value (Vanhaecke *et al.*, 1990). One gram of cyst material can yield 0.4456 g of naupliar dry weight or 0.730 g of decapsulated cyst dry weight (Vanhaecke & Sorgeloos, 1983).

The use of mixed dry/live diets in larviculture

Many species of marine and freshwater fish larvae ingest formulated feeds but the associated poor growth is thought to be due to lack of digestion or assimilation of these feeds (Holt, 1993). For example, an increase in the dry food component of a mixed dry/live diet caused an increase in the percentage of malformed European minnow larvae, *Phoxinus phoxinus* (Kestemont & Stalmans, 1992). Similarly, a high rate of malformation was found in carp larvae, *C. carpio*, fed a commercial dry diet, and this was corrected through the supplementation of *Artemia* nauplii. In another example, a continuous supply of live food such as *Artemia* to common carp larvae decreased the incidence of cannibalism (Von Lukowicz (1976) cited in Kestemont, 1995) but the low rate of cannibalism may also have been the result of variation in growth in those larvae fed on dry food alone (Kestemont, 1995). Thus, dry food in the diet of some fish species reduced growth and development and this was only corrected through supplementation with live food. However, Kestemont (1995) achieved the best growth in goldfish larvae by feeding them a mixed diet of *Artemia* and dry food during the first weeks of growth.

Problems associated with feeding dry diets include deterioration of water quality and tank cleanliness (Appelbaum & Uland, 1979; Sharma & Chakrabarti, 1999) and the rapid proliferation of micro-organisms (Charlon & Bergot, 1984). However, commercial dry diets are more convenient to feed than live food and do not require labour-intensive facilities for live food production (Rottmann *et al.*, 1991). Mixed diets can be chosen to match the nutritional requirements for larvae and provide a back-up food supply in case of production failure, and they can be used to reduce the weaning period of juvenile fish onto artificial diets (Rottmann *et al.*, 1991). For example, the difficulty of getting cultured cyprinids to accept dry starter diets can often be overcome by feeding a mixed live/dry food

diet initially and then weaning the fish onto dry food after 1-2 weeks (Opuszynski & Shireman, 1993). Good growth and high survival of goldfish reared under intensive conditions has been achieved with a mixed diet of *Artemia* and dry food (Kestemont & Mélard, 1991).

Size variation

Size variation is a major cause of cannibalism and other agonistic behaviour among fish larvae. The primary causes for large size variation, other than genetic differences between siblings, are food availability and feeding frequency (Hecht & Pienaar, 1993), a lack food items of the right size and type (Wankowski & Thorpe, 1979) and low feeding levels (Wickins, 1985; Abi-Ayad & Kestemont, 1994; Goldan *et al.*, 1998). In commercial culture systems, fish are routinely size-graded in order to overcome the problems associated with high size variation. The process of size-grading is stressful to the fish, labour intensive and needs to be done regularly (Goldan *et al.*, 1998).

Size-grading of fish has advantages in that small fish grow better when their larger siblings are removed due to the reduction of competition and social hierarchy (Gunnes, 1976). It is best to maintain fish of similar size in the culture tanks to improve the utilisation of food. Gunnes (1976) found that size-grading at an early age resulted in better growth than at a later stage when social structures may have already developed. One aim of this research is to test the effect of size variation of diet type, tank design and feeding rate.

Feeding and agonistic behaviour in larval fish

Fish depend on their sensory capacities to locate food (Kestemont & Baras, 2001). Once located, their ability to capture and ingest food items is important to successful aquaculture.

Additionally, the fish's physiological capacity to ingest and transform the ingested nutrients and the effect of various environmental factors (Figure 1.1) will determine growth performance (Kestemont & Baras, 2001).

In general, capture success, i.e., the percentage of successful feeding strikes, is low during first feeding in fish larvae but rises rapidly during early development (Houde & Schekter, 1980). Capture success improves with increasing feeding experience (Colgan *et al.*, 1986) and improved manoeuvrability (Hurst, 1994). No study is known to have recorded prey capture and ingestion or rejection rates for goldfish larvae. This study compares preference for *Artemia* nauplii or cysts in goldfish larvae from three days after hatching to 17 days after hatching.

The results of feeding trials that compare different diets and different feeding levels can be used to understand reasons for high size variation in goldfish larvae. Size variation is a primary cause of agonistic behaviour, which in turn affects rates of cannibalism and so can be viewed as both a cause and effect of cannibalism (Hecht & Pienaar, 1993). Cannibalism can be regarded as an alternative feeding strategy adopted by many carnivorous larvae and early juveniles when resources become limiting (Hecht & Pienaar, 1993).

Agonistic behaviour such as chasing and nipping the body and fins of conspecifics can be prompted by a number of factors other than size variation. For example, larval density has a greater effect on aggressive and non-aggressive behaviour of snapper larvae *L. argentimaculatus* than food availability (Hecht *et al.*, 1996). This has also been shown for koi carp, *C. carpio*, where aggression and cannibalism increased with larval density (Van Damme *et al.*, 1989). In African catfish *Clarias gariepinus* aggression and cannibalism

decreased with an increase in larval density and increased in relation to decreased food availability (Hecht & Appelbaum, 2001).

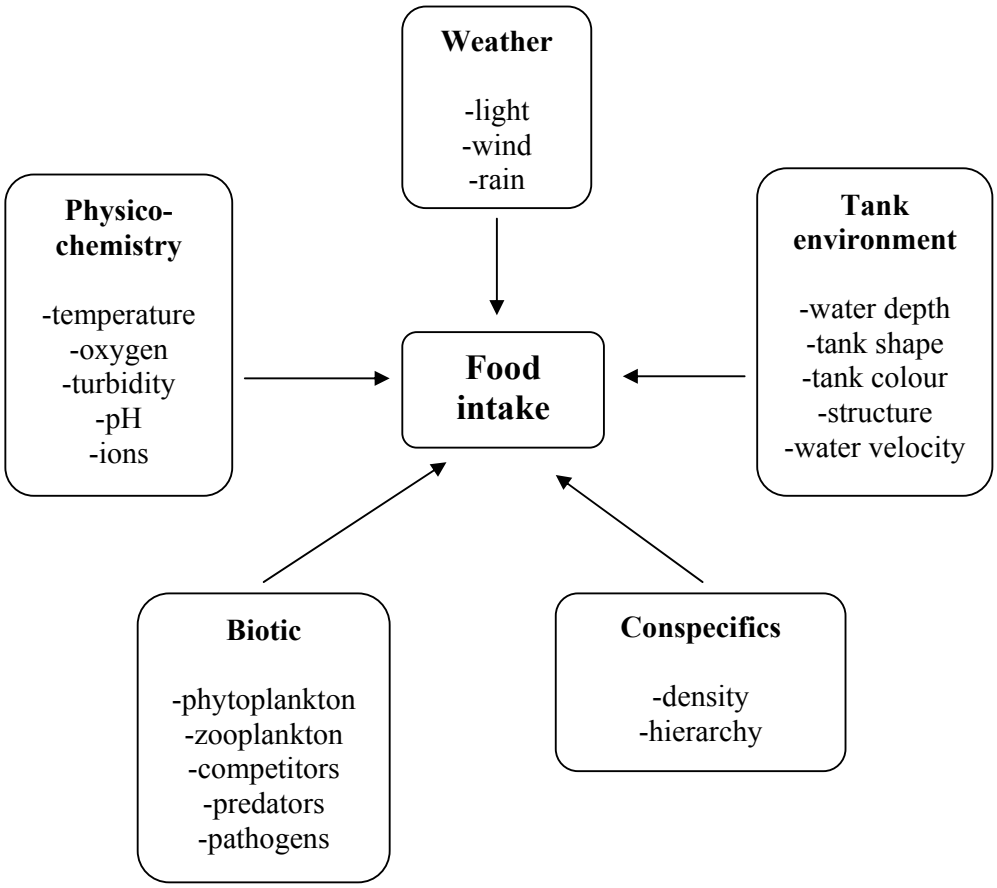


Figure 1.1 Overview of environmental influences on feed intake in fish. Interactions between factors are not shown (Adapted from Kestemont & Baras, 2001).

Goldan *et al.* (1997) suggest four mechanisms related to social rank that affect the growth of fish: (1) direct competition for food when larger fish consume more food by defending the food source where possible; (2) social stress caused by the agonistic behaviour of larger fish; (3) increased motor activity where subordinates expend energy evading dominant fish; and (4) a dominance cost whereby subordinate fish grow better since they do not expend energy in being aggressive. Aggressive individuals monopolise food supplies, and by

diverting their attention from the food source to chasing competitors, they allow less aggressive fish to obtain food and gain a growth advantage (Ruzzante, 1994).

Prey selection

Visual acuity, visual threshold and spectral sensitivity, prey contrast, shape, and mobility are known to affect prey selection in fish larvae (Holm, 1986; Khadkar & Ramakrishna Rao, 1986; Cunha & Planas, 1999). Shim and Bajrai (1982) state that intake of food in juvenile guppy *P. reticulata* is determined by particle size with larger fish eating larger prey. In this study, differences in preference for either decapsulated cysts, which are small and move slowly in the water current, and *Artemia* nauplii which are larger and more mobile, will be examined according to ingestion and rejection rates. The results will be compared with data for other fish species. Gulbrandsen (1993) and Mookerji and Rao (1993) found that although larval halibut *H. hippoglossus* L. were non-selective during their first two days of feeding, fish became more selective as they grew. Larvae will preferentially capture larger prey at both high prey densities and at low larval population densities (Opuszynski & Shireman, 1993). Indiscriminate prey capture is energetically the best strategy at low prey densities as the energetic cost of swimming in a selective feeding pattern outweighs the benefit of capturing more suitable prey items.

The ability to select prey varies with prey density (Emlen, 1966; Chakrabarti & Jana, 1991; Schael *et al.*, 1991; Opuszynski & Shireman, 1993), prey distribution (Jakobsen & Johnsen, 1987; Chakrabarti & Jana, 1991, light intensity (Mills *et al.*, 1986; Mookerji & Rao, 1993), predator size (Mills *et al.*, 1986), experience (Ringler, 1979), hunger level (Kislalioglu, 1976), and competition (Rubinstein, 1981). This study will test the effect of feeding

experience, predator and prey size and mobility, and intra-specific competition of goldfish larvae on feeding behaviour.

Preservation and shrinkage

Preservation of larvae in formalin results in shrinkage or damage that complicates the use of morphological measurements (Puvanendran & Brown, 1999). For example, preservation of fish larvae in formaldehyde has been shown to increase the stiffness of the fish larvae and measurements should therefore be made on unpreserved specimens (Arts & Evans, 1987). In larval capelin *Mallotus villosus* preservation in either 5% buffered formalin or alcohol (100% anhydrous) resulted in significant shrinkage over time with a significantly greater shrinkage in alcohol (Kruse & Dalley, 1990). The time that the larvae remained in the preservation media ranged form thirty minutes to 24 weeks. This makes comparison with this study difficult as it examined shrinkage over the course of 36 hours. A preliminary study on shrinkage in formalin was conducted to determine the extent of shrinkage. This knowledge is important to ensure the accuracy of measurement required for this study.

Research objectives

The research objectives for this study are to:

1. Determine if and to what extent shrinkage occurs in goldfish larvae over 36 hours of preservation in a 10% formalin solution;
2. Compare growth and survival in goldfish larvae fed either *Artemia* nauplii, decapsulated *Artemia* cysts, or a mixed live/dry diet;
3. Explore the effect of diet on the development of goldfish larvae and juveniles to determine how larval plasticity is affected by diet type.

4. Determine the effect of suspending *Artemia* cysts in the water column (for the first 62 days after hatching) on growth, survival and size variation of larvae.
5. Optimise the feeding level of decapsulated *Artemia* cysts for goldfish larvae and juveniles for the first 16 days after hatching in order to maximise growth and survival;
6. Observe and quantify feeding behaviour and agonistic behaviour of goldfish larvae and juveniles as well as determine if and how behaviour changes as fish grow.

Thesis outline

Chapter 1 (Introduction) presents an overview of the literature on larval rearing, development and feeding behaviour related to the aims of this study.

Chapter 2 (General materials and methods) presents experimental and statistical methods which are common to most chapters and provides details of the recirculating experimental system and tank design. It describes and illustrates the device used for measuring mouth-gape. It also presents details of the environmental conditions under which the fish were reared and how data were collected.

Chapter 3 (Larval shrinkage in formalin over 36 hours and inter- and intra-measurer variation) deals with shrinkage in formalin-preserved goldfish larvae and a comparison of inter- and intra-measurer error. The discussion compares the findings with existing literature and makes recommendations on how measuring needs to proceed in the experimental work for the following chapters.

Chapter 4 (Growth and development of larval goldfish fed either *Artemia* nauplii, a mixed live/dry diet, or decapsulated *Artemia* cysts) examines the potential for using decapsulated *Artemia* cysts as an alternative to *Artemia* nauplii or mixed live/dry diets in goldfish larval rearing. Growth and survival of fish fed cysts is compared to that of those fed nauplii or a mixed diet with respect to growth performance, survival and size variation. It presents an overview of studies on morphological plasticity in fish larvae and relates them to the results of this study.

Chapter 5 (Growth and development of larval goldfish fed *Artemia* cysts that either sank to the tank bottom or were kept suspended in the water column) deals with the problem of decapsulated cysts sinking to the bottom of the tank where they are potentially less accessible to goldfish larvae. Growth, survival and size variation of larvae reared in flat-bottomed tanks was compared with that from V-shaped tanks over the first 16 days after hatching. An additional treatment was included to record growth and survival until 62 days after hatching.

Chapter 6 (The effect of feeding rate of decapsulated *Artemia* cysts on larval and juvenile goldfish growth and survival) evaluates larval goldfish growth performance at different feeding levels and aims to optimise the number of cysts required to feed larval goldfish in order to best utilise the food.

Chapter 7 (Larval and juvenile preference for either decapsulated *Artemia* cysts or *Artemia* nauplii: ingestion and rejection patterns according to diet type) presents results of

behavioural observations of fish at different ages with emphasis on prey preference and frequency of agonistic behaviour.

Chapter 8 (General discussion) critically links results and arguments made in Chapters 3-7 and makes recommendations for feeding larval goldfish and discusses larval development under different culture conditions.

Chapter 2

General materials and methods

Origin of the experimental fish

Larval goldfish were obtained from the freshwater tunnel system at the Department of Ichthyology and Fisheries Science (DIFS), Rhodes University. Mature goldfish were conditioned for three weeks in a 2.5-m^3 tank, which was part of a 25-m^3 recirculating system situated in the greenhouse tunnel. Approximately 15 male and 15 female fish were injected with Aquaspawn® (Spawnrite Ltd.) to induce spawning, and eggs were collected early the following day. Once these eggs had hatched the larvae were transferred to the experimental aquarium system.

System design and management

The closed recirculating system (Figure 2.1) used for all experiments had a total water volume of 800 L. Water leaving the experimental tanks passed though a trickling filter and two 90-L submerged biological filter boxes containing shredded plastic filter media. Oyster shells were placed in meshing inside the pump-sump to stabilise pH. A 0.3 KW submersible pump circulated the water through the system and about half the flow was directed back into the sump. Water temperature was thermostatically maintained at 23 ± 1.5°C by means of a 0.6 KW submersible heater. This temperature is within the temperature range suitable for larval goldfish growth (Abi-Ayad and Kestemont, 1994; Wiegand *et al*., 1988). Photoperiod was maintained at 12L :12D by L18 W/72 Biolux® tubes (Osram, Germany), each one-meter tube suspended across three experimental fish tanks.

The experimental system comprised 18 glass aquaria (22.5 cm x 30 cm x 23.4 cm; water depth 20 cm) each filled to a volume of 13 litres (Chapters 3 and 4) and a volume of 10 litres (Chapters 5, 6, and 7; Figure 2.2). Each experimental tank was stocked at 12 fish /L with the exception of the behavioural study where the stocking density was 25 fish /L (Chapter 7). All treatments were replicated twice. Water was supplied to each tank via two inlet pipes. The flow rate of water to the tanks was set to achieve approximately three exchanges per hour and water flowed out of the tanks through an outflow covered with 150-μm mesh into a gutter leading into the trickling filter. The water in the sump was aerated before being distributed to the tanks to avoid the need for air-stones in each tank. Aquaria were randomly assigned to treatments and replicates to eliminate positional bias.

Water quality was measured at the beginning and end of each study. Ammonia (NH_4^+) and nitrite (NO_2^-) were measured using aquarium test kits (Interpet, South Africa), and pH was measured using a portable pH meter (Hanna, USA). Ammonia and nitrite remained between 0-0.1mg/L throughout the experimental period and pH ranged from 8.0 to 8.1.

Feeding

Grade A *Artemia* cysts (Bio-Marine Brine Shrimp Eggs, batch AA05259, Hawthorne, California, USA) were decapsulated according to the method described by Lavens and Sorgeloos (1996) and fed as cysts or Instar I nauplii depending on the experimental design of the respective studies. The dry food component, AquaNutro Goldfish Food (WPK, Malmesbury, South Africa), was crushed and sieved to a particle size of 125-212 μm. The dry diet contained 45 % crude protein, 6 % crude fat, 3 % crude fibre, 26 % nitrogen-free extracts, and 9 % ash according to the supplier's specifications.

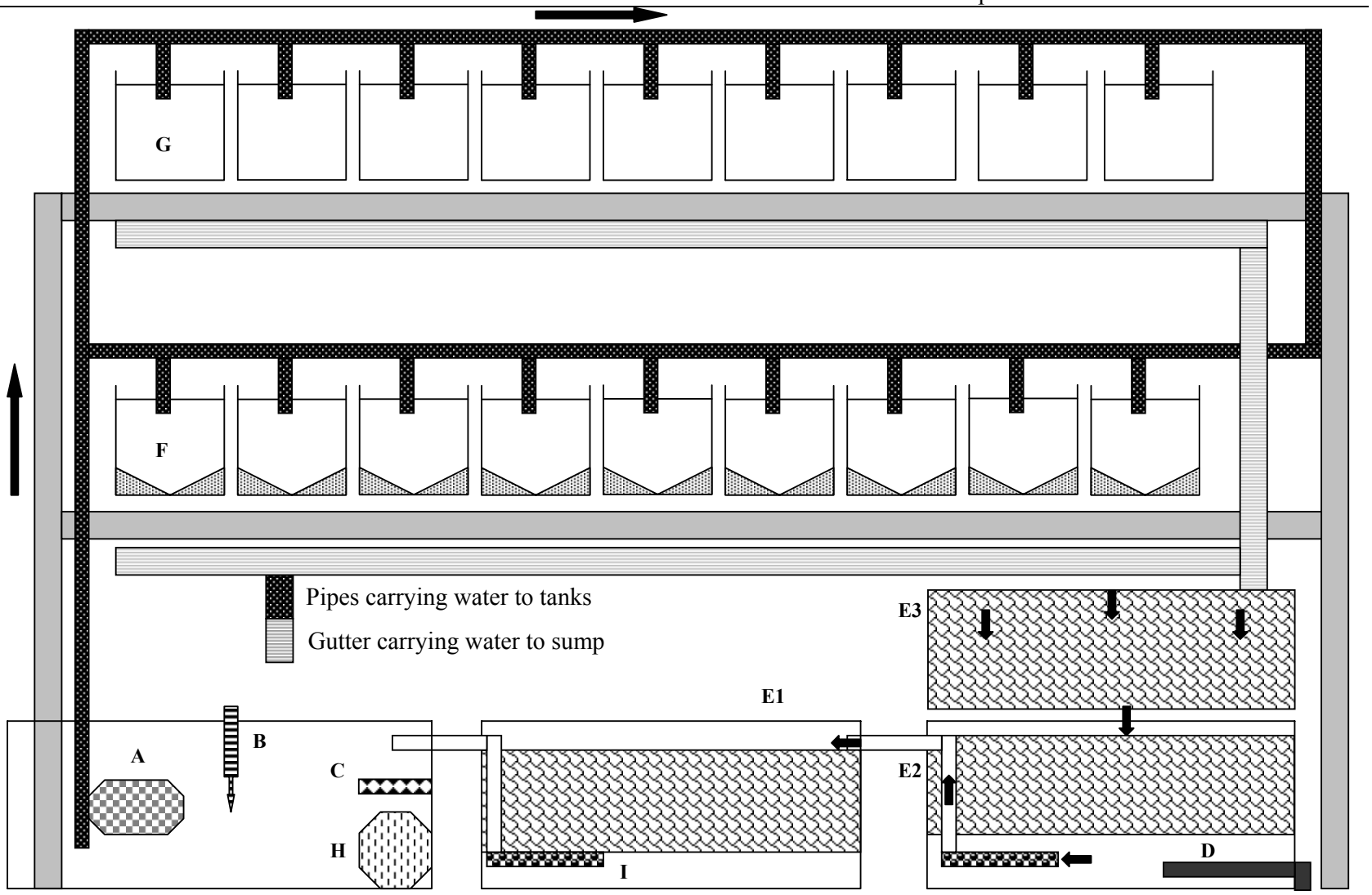


Figure 2.1. Experimental glass aquarium system (arrows show direction of water flow). Pump (**A**); thermostat (**B**); air-stone (**C**); Heater (**D**); biological filters (**E1, E2**); trickle filter (**E3**); tank set-up as described for chapters 5, 6 and 7 (**F**); tank set-up as described for chapters 3 and 4 (**G**); Oyster shell buffer (**H**); outflow pipe from filter box (**I**).

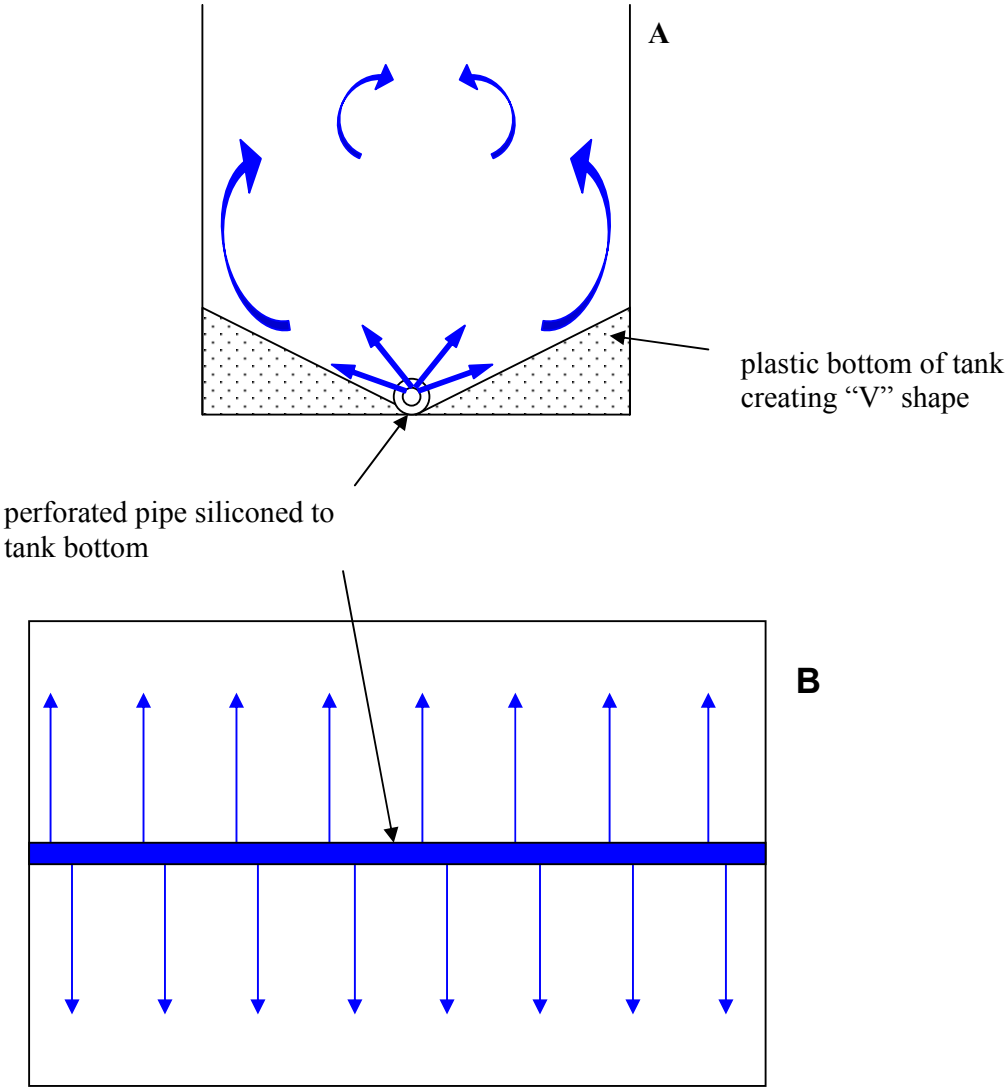


Figure 2.2. Diagram illustrating V-shaped design of the tank bottom used for experiments described in Chapters 5, 6 and 7. Arrows show water movement; front view (**A**); top view (**B**).

The amount of food, both live and dry, available to the fish at each feeding was kept constant but the total amount per tank decreased in proportion to the estimated number of fish remaining after daily sampling. Hatched *Artemia* nauplii were concentrated in one litre of seawater and the rehydrated and decapsulated *Artemia* cysts were stored in one litre of freshwater. To determine the number of *Artemia* nauplii and cysts to be fed each day, five

1-ml aliquots of the water containing either nauplii or cysts were counted in a 1-ml pipette tube. The average number of nauplii or cysts per millilitre of water was calculated, and the number of prey items to be fed to each experimental tank was determined by multiplying the number of fish in the tank by the required number of prey items per fish. The required volume of water containing nauplii or cysts to be added to the tank was calculated in the following way:

$$\frac{\text{(Required number of prey items)}}{\text{(Number of prey items in 1ml of water)}} = \text{ml to feed}$$

A 1-ml graduated pipette was used to measure the volume of water and prey items onto a 100 μm screen that allowed the saltwater to pass through it. This screen was then rinsed in the experimental tank to release the food items. This was done to avoid salinity build-up in the experimental system.

Fish were fed five times per day at two-hourly intervals (08:00h; 10:00h; 12:00h; 14:00h; 16:00h) until day seven, and three times per day, every four hours (08:00h; 12:00h; 16:00h) until the end of the study. Faecal matter and uneaten food were siphoned off the bottom of the tanks each morning before first feeding after which fish were removed for measurements. Once a day, dead fish were removed and counted.

Sampling of fish

In all experiments, with exception of the behavioural studies (Chapter 7), five fish were randomly netted from each tank every morning before first feeding. These fish were pooled according to treatment and the sequence in which the treatments were sampled was randomised. A pilot study investigated the effect of formalin shrinkage over time (Chapter

3), and although some shrinkage occurred over the 5-hr period needed to measure the samples, this error fell within the range of errors incurred by the measurer. Thus, all fish were measured as fast as possible after they had been placed on ice.

Data collection

Total length, standard length/notochord length, head length, body length, tail length, body depth, and head depth (Figure 2.3) were measured for each fish before the measurement of mouth-gape (Chapters 3-6). For the first two days, fish were measured using an ocular eyepiece under a dissecting microscope. From day three, fish were measured under the dissecting microscope using electronic vernier callipers. A previous experiment determined that there was no significant difference between the two measuring techniques. The time at which metamorphosis occurred was noted (Chapters 5-7).

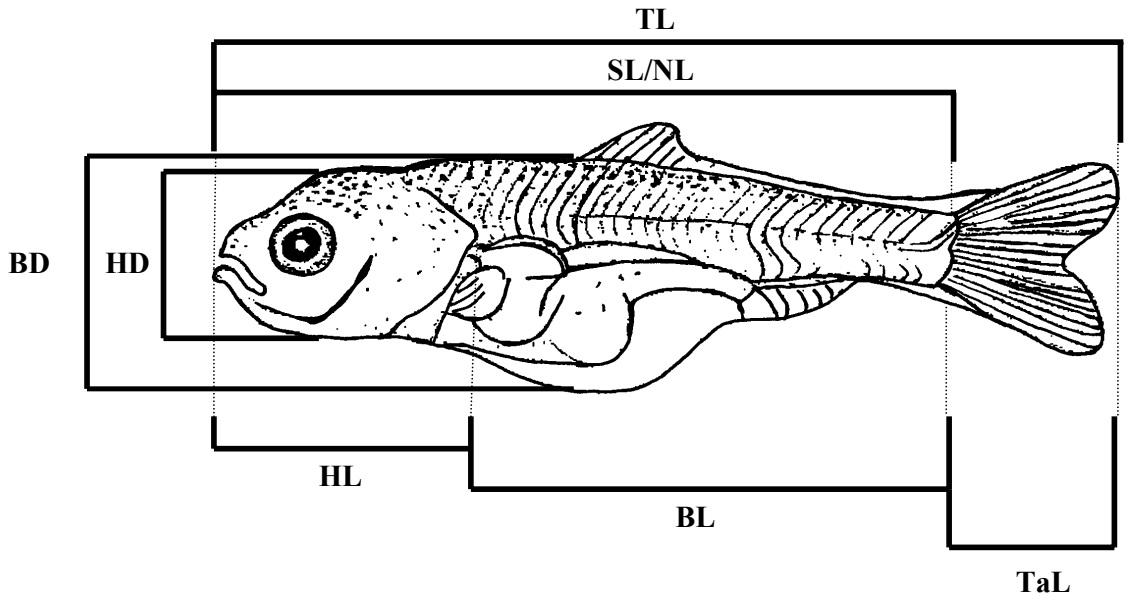


Figure 2.3. 12-day old goldfish larvae (TL 13.8 mm) indicating measurements taken. Total length (**TL**); standard length (**SL**) or notochord length (**NL**) in pre-metamorphic larvae; body depth (**BD**); head depth (**HD**); head length (**HL**); body length (**BL**); tail length (**TaL**). All measurements are in mm.

A pilot study was conducted to test the accuracy of the measuring methods and to determine the variation in results from different measurers. The results (Chapter 3) showed that there were no significant differences between measurers or methods tested.

Measuring mouth-gape

Mouth-gape was measured using a precision micrometer device described by Arts and Evans (1987) and modified by Terry Longman (DIFS, Rhodes University) (Figure 2.4 and Figure 2.5). This method had been found to be more accurate than using vernier callipers (Arts and Evans 1987).

The gape micrometer consists of three basic components: Plexiglas body; micrometer scale with attached head (flat disk); and exchangeable stainless steel rods with conical tips over which the fish's mouth will fit. Screws clamp the steel rods in place. A range of differently sized steel rods was machined allowing the measurement of mouth-gape from 0.05 – 2 mm and the two rods used were able to measure in the range of 0.05-1.5 mm and 0.5-2 mm (Figure 2.5).

Calibration of the micrometer

The micrometer was set to zero and the tips of the measuring cones were aligned with the flat surface of the micrometer head. This was done by using a glass microscope slide and pressing it firmly against the flat plate on the zeroed micrometer, and then moving the conical point of the rod until it touched the slide (Arts and Evans, 1987).

Under a dissecting microscope, the fish was held in the clamp (Figure 2.4) in such a way that the head was free, and the mouth was slipped over the conical end of the measuring rod until resistance was encountered. Flaring of the gills or distortion of the head meant that the cone was pushed too far into the fish's mouth which would not give an accurate measurement of the mouth-gape. Once the fish was in position on the cone, the micrometer was adjusted until the flat disc was directly opposite the corner of the upper and lower jaw. The measurement obtained from the micrometer (**X**) was the perpendicular distance that the cone reached into the mouth and, using trigonometry, the following formula was used to calculate the diameter of the cone at this point:

$$\text{Mouth-gape} = (\mathbf{X}) \cdot \tan 20° \cdot 2$$

where **X** was the micrometer reading and 20° was the angle of the cone used.

Mouth-gape was not measured earlier than 4 days after hatching (DAH) which was day two of the experiment, because before this time the fish were too small for the smallest available measuring cone.

Statistical analysis

All data were tested for normality using the Kolmogorov-Smirnov test and for equality of variance using Lévene's test. If data were found to differ significantly from the normal distribution or if they showed unequal variance, then non-parametric tests were used to test for differences.

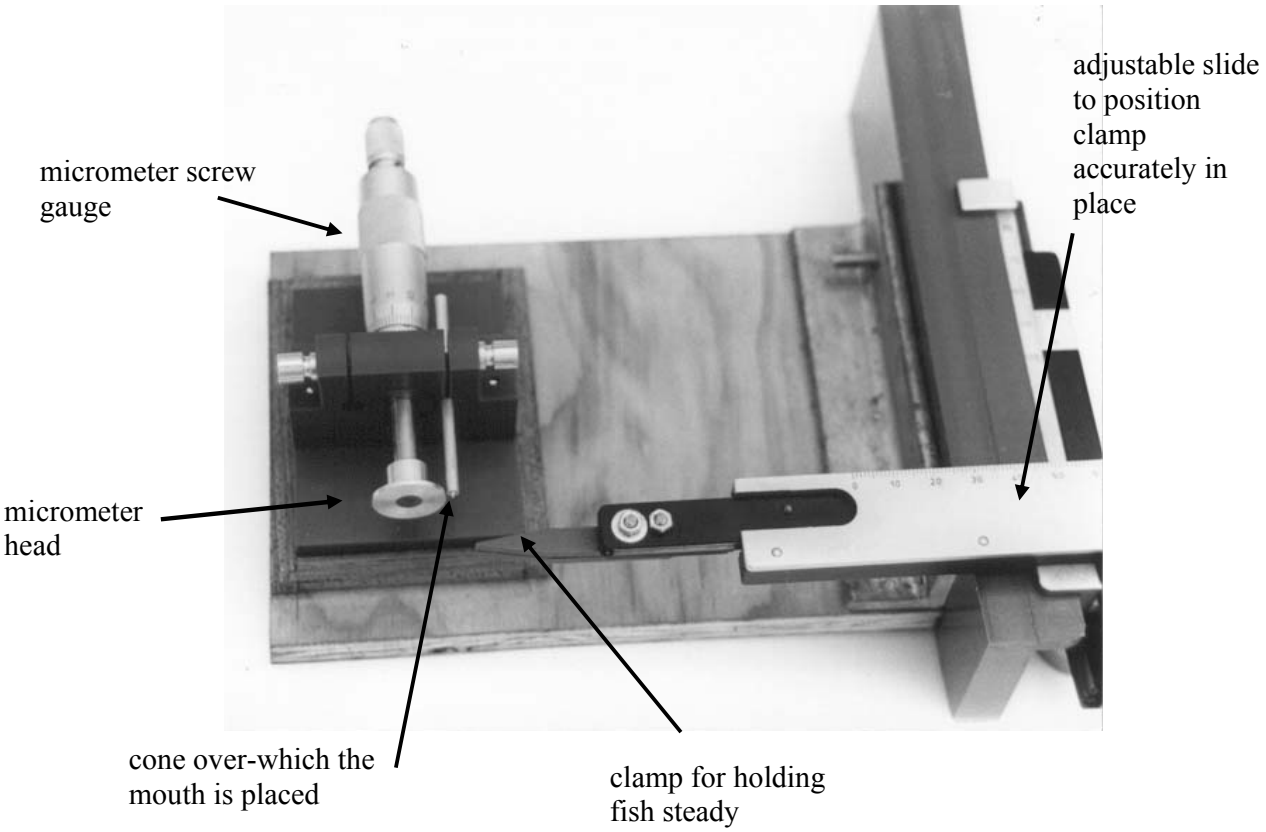


Figure 2.4. Photograph of precision micrometer device used for measuring mouth-gape.

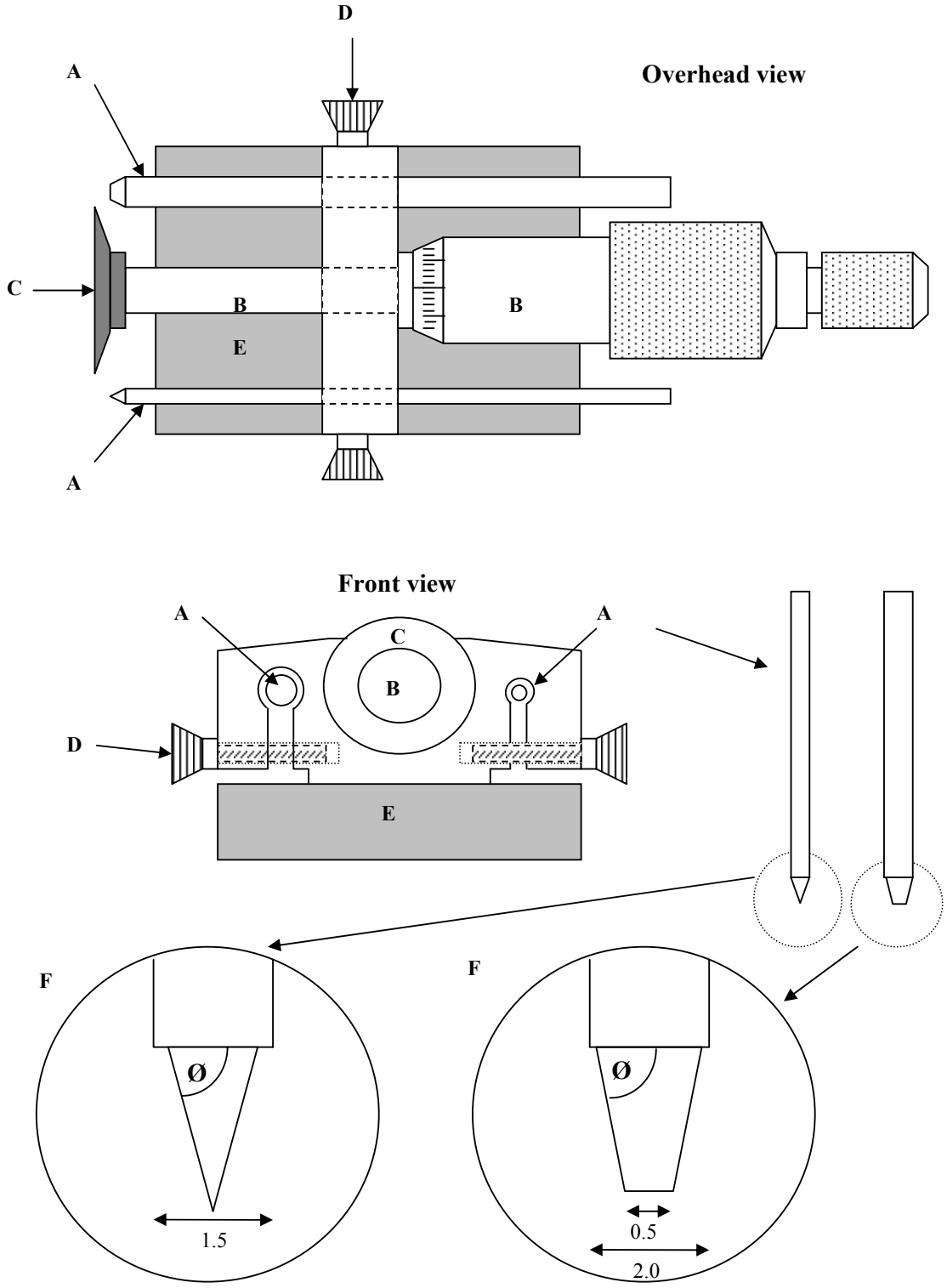


Figure 2.5. Overhead and front views of the gape micrometer. Stainless steel rods (**A**); micrometer body (**B**); micrometer head (**C**); Screw to clamp rods in place (**D**); Plexiglas body (**E**). Detail of the small stainless steel rods used where **Ø** is 20° (**F**). Measurements are in mm.

Chapter 3

Larval shrinkage in formalin over 36 hours and inter- and intra-measurer variation in data recording

i) Formalin shrinkage

Introduction

In order to understand and describe growth in larval fish, it is necessary to take accurate measurements of various morphological characteristics. Larvae are usually preserved in some kind of fixative before being weighed or measured. Denaturation and dehydration of the fish body resulting from fixatives, such as formalin or alcohol makes it difficult to obtain accurate morphometric values (Takizawa *et al*., 1994) and shrinkage varies from species to species (Table 3.1). Kruse and Dalley (1990), found that preserved capelin (*Mallotus villosus*) larvae shrank at different rates when preserved in either formalin or anhydrous alcohol and that there was differential shrinkage with total length decreasing at a faster rate than standard length. They also found that there was significant shrinkage after 30 minutes but no further shrinkage after 24 weeks. This early shrinkage motivated this study as it was planned that fish larvae would be stored in formalin prior to measurement.

It has been shown that alcohol fixation resulted in faster and greater shrinkage than formalin fixation in fish larvae with smaller fish being affected more than larger fish (Radtke & Waiwood, 1980; Fowler & Smith, 1983; Kruse & Dalley, 1990; Takizawa *et al*., 1994). It was therefore necessary to investigate the rate of shrinkage of goldfish larvae in formalin in

order to determine if correction factors needed to be used to compare the pre-fixed measurements to measurements taken from preserved larvae.

Table 3.1 Summary of findings on shrinkage in fish larvae after fixation. Values in parentheses show time in days and all length values are total length. **BW** represents body weight.

Species	Fixative	%Shrinkage	Fish size (or weight)	Author
Dicentrarchus labrax	4% formalin 70% ethanol	6.5% (6) 6.7% (6)	<10mm	Jennings, 1991
Paralichthys lethostigma	35‰ salt 10% formalin (in seawater)	5.2-9.4% (7yrs) 5.6-7.8% (7yrs)	±11.27mm	Tucker & Chester, 1984
Clupea harengus	4% formalin 10% formalin 20% formalin	15.5% BW (10) 3.5% BW (10) 0.3% BW (10)	210.7μg	Hay, 1984
Merluccius bilinearis	4 % formalin (in seawater) 95 % ethanol	3.4 % (15) 4.8 % (15)	4-15mm	Fowler & Smith, 1983
Pagrus major	10 % formalin	1-16 % (15) 2-31 % BW (15)	25.64mm	Takizawa *et al.,* 1994
Mallotus villosus	5 % formalin 100 % anhydrous alc.	2.9 % (1) 9.5 % (1)	10.3mm	Kruse & Dalley, 1990

The null hypothesis stated that there will be no significant shrinkage in total length and body depth due to fixation in 10 % formalin. The alternative hypothesis stated that there will be a significant difference between pre-fixed and preserved specimens in total length and body depth due to fixation in 10 % formalin.

Methods and materials

Fifteen larval goldfish were netted from the experimental glass aquarium system (Chapter 2) three days after hatching. Larvae were placed into individual glass vials which were then put into iced water. Total length and body depth were measured using a dissecting microscope with an ocular eyepiece. Immediately after the measurement the fish were transferred into a 10% formalin solution (pH 4.3). Total length and body depth was measured repeatedly from each fish after it had been fixed in formalin for 6 h, 12 h, 24 h and 36 h.

The independent variable was time of immersion in formalin and the dependent variables were total length and body depth. Shrinkage in both dependent variables was tested for difference using a student's t-test. Data were tested for normality (Kolmogorov-Smirnov: $p > 0.05$) and equality of variance (Levene's test: $p > 0.05$). Differences between the pre-fixed measurements and the sequential measurements taken over time were tested by applying analysis of co-variance (ANCOVA) to test for differences between the slopes of the regression models for body depth and total length as a function of time, respectively.

Results

Total length shrinkage due to formalin (Table 3.2) was significant ($p \leq 0.0001$) with fish losing 1.18 mm within 36 hours on average, which amounted to a 17 % decrease within this period. The decrease in body depth was also significant ($p \leq 0.003$) as the larvae lost on average 0.16 mm or 14 % of their unfixed body depth within 36 hours. No significant difference was found between the rates of shrinkage of total length and body depth over 36 hours ($F_{1,116} = 2.76$; $p \geq 0.05$).

The regression models and correlation coefficients (r^2) for shrinkage over 36 hours in 10 % formalin are (x is time in days):

Preserved total length/pre-fixed total length = 0.992-0.004*x (r^2 = 45 %)

Preserved body depth/pre-fixed body depth = 0.996-0.003*x (r^2 = 32 %)

Table 3.2 Accumulated percentage loss in mean total length and mean body depth showing actual measurements ± standard deviation (SD) for larval goldfish with different exposure times to formalin.

Time (hours)	Accumulated % loss in total length	Mean total length (mm) ±SD	Accumulated % loss in body depth	Mean body depth (mm) ±SD	Sample size
0	0	6.93 ± 0.40	0	1.13 ± 0.11	15
6	4.6	6.61 ± 0.46	0.6	1.13 ± 0.07	15
12	7.0	6.45 ± 0.47	7.7	1.04 ± 0.09	15
24	10.9	6.18 ± 0.47	6.4	1.06 ± 0.11	9
36	17.0	5.77 ± 0.34	14.0	0.97 ± 0.09	9

ii) Measurement error for mouth-gape

Ten larval goldfish were removed from the experimental glass aquarium system (Chapter 2) seven days after hatching. The fish were netted randomly and placed into a labelled Eppendorf tube, which was placed in iced water. Mouth-gape was measured for each fish according to the methods described in Chapter 2 and then measured again by the same measurer. A second measurer then measured the same fish and then fish were mixed together and their mouth-gapes measured.

The null hypothesis stated that there will be no differences between measurements taken by the same measurer and between measurements taken by different measurers, respectively. The alternative hypothesis therefore, is that there will be differences between the same measurer's results and between two different measurers. Mean values were tested for difference using a pair-wise student's t-test after normality (Kolmogorov-Smirnov: $p < 0.1$) and equality of variance (Levene's test: $p > 0.05$) were tested.

Results

There was no significant difference ($p = 0.99$) between the different measurements taken indicating that this method of measuring mouth-gape is repeatable.

Table 3.3 Mean mouth-gape measurement ± standard deviation (SD) of fish measured repeatedly by experimenter **(A)** and an independent measurer **(B)** and then again randomly by **(A)** again. No significant difference was found between any of the measurements taken ($p > 0.05$; $n = 10$).

Measurer	Mouth-gape (mm)±SD
(A) first measurement	0.653±0.332
(A) second measurement	0.641±0.313
(B) independent measurer	0.654±0.334
(A) random order	0.654±0.324

Discussion

There was no significant difference in the rate of shrinkage between total length and body depth during the first 36 hours of fixation in 10 % formalin. This is contrary to the findings of Kruse & Dalley (1990) and Takizawa *et al*. (1994), who found differential shrinkage between total length, standard length and weight. In the 15-day study by Takizawa *et al*. (1994) and the 24-week experiment by Kruse & Dalley (1990) shrinkage was not quantified for the first 36 h. From the results of this study, total length and body depth can be measured reliably during the first 36 hours of fixation in a 10 % formalin solution and a single correction factor can be used to estimate the prefixed values. However, as other morphometric measurements than those tested here were taken in subsequent experiments, larvae and juveniles were measured immediately after sampling to avoid potential error.

Shrinkage varies considerably between species and between differently sized individuals of the same species (Table 3.1). Degree of shrinkage depends primarily on the osmotic strength of the solution and un-buffered 4 % formalin in freshwater would give the most

accurate length measurements (Tucker & Chester, 1984). Water loss is one of the factors responsible for shrinkage in fixatives and the varying moisture content within the body of the fish can cause differential shrinkage. Larger fish have a lower percentage water in their bodies than smaller fish (Hay, 1984). Thus, percentage shrinkage is greater in small fish (Takizawa *et al.,*1994). The measurement of mouth-gape was shown to be accurate and repeatable using the methods applied here.

Chapter 4

Growth and development of larval goldfish fed either *Artemia* nauplii, a mixed live/dry diet, or decapsulated *Artemia* cysts

Introduction

The changeover from endogenous to exogenous feeding is a critical time for fish larvae and the availability of food of a suitable size is therefore essential for commercial rearing (Appelbaum & Uland, 1979; Gulbrandsen, 1993; Jähnichen & Kohlmann, 1999). Food size and type may influence larval development and growth and may lead to different rates of morphometric change (Mills *et al*., 1996). In order to accurately predict the right particle or prey size and type of food required for fast growth it is important to know the mouth-gape size and the relationship between mouth-gape and total length or standard length.

Mouth-gape determines the maximum size for potential prey items and this has been shown in several species of fish larvae such as grass carp *Ctenopharyngodon idella*, silver carp *Hypophthalmichthys molitrix*, bighead carp *Aristichthys nobilis* (Dabrowski & Bardega, 1984), and red snapper *Lutjanus argentimaculatus* (Doi *et al*., 1997), which became cannibals when food was scarce. Measurement of mouth-gape allows researchers to estimate the efficiency with which fish ingest prey of different size (Arts & Evans, 1987; Cunha & Planas, 1999). For example, Shirota (1970) showed differences in the ratio of mouth gape to total length between species and hypothesised that fish grew better with increasing mouth-gape to length ratios. To contribute to this topic, the development of the ratio of mouth-gape to total length will be investigated for different feed types.

Initially, the larval gut is relatively simple in many species and the stomach is not fully functional (Holt, 1991). Goldfish larvae rely heavily on the presence of proteolytic enzymes in their food for digestion and live foods such as *Artemia* nauplii supply these enzymes (Mills *et al*., 1996). In addition, fish zymogens have been shown to become activated by invertebrate enzymes (Dabrowski, 1991; Abi-Ayad & Kestemont, 1994; Mischke & Morris, 1998). Growth of fish fed one of three diets, *Artemia* nauplii, mixed live/dry diet or decapsulated *Artemia* cysts will be compared and results will have practical applications to larval goldfish rearing.

Artemia nauplii as a diet for fish

Artemia nauplii are used as a diet for rearing the larvae of many marine food fish species and their application to freshwater fish larviculture has been shown (Bryant & Matty, 1980; Sorgeloos *et al*., 1991; Dhert *et al*., 1997). Instar I *Artemia* nauplii consume their own energy reserves and can develop into instar II nauplii within 6 to 8 hours. It is therefore important to feed instar I nauplii to the fish rather than starved and transparent instar II meta-nauplii which are less visible to the larvae (Dhert *et al*., 1997). Hatching *Artemia* from cysts is time-consuming and often a hatchery is required to facilitate a continuous production of nauplii. Thus, it would be beneficial to find an alternative diet that would require less labour, time and money to prepare.

Decapsulated cysts as a substitute for live Artemia

Decapsulated *Artemia* cysts have several advantages over *Artemia* nauplii as a larval diet in freshwater aquaculture. Cysts (1) are more nutritious due to their high level of highly unsaturated fatty acids (HUFA's), amino acids and energy content; (2) can be prepared without the need for a hatchery; (3) can be stored in a fridge for years if dehydrated; (4)

have a smaller diameter which enables them to be ingested by smaller fish (Verreth *et al*., 1987); (5) persist for longer in freshwater than nauplii; and (6) do not leach nutrients (Vanhaeke *et al*., 1983; Vanhaecke *et al*., 1990; Dhert *et al*., 1997).

Cysts are therefore a good alternative to live *Artemia* as a diet for larval fish and this has been confirmed for many species. For example, the larvae of several freshwater fish species including grass carp *C. idella*, common carp *Cyprinus carpio* (Jähnichen & Kohlmann, 1999), ide *Leuciscus idus*, and barbel *Barbus barbus* (Vanhaecke *et al*., 1990) grew equally well or better when fed decapsulated cysts compared to those fed a commercial starter feed. Furthermore, growth and survival of guppies *Poecilia reticulata* was significantly better among fish fed decapsulated cysts than in fish fed *Moina* (Dhert *et al*., 1997). In larvae of *C. idella* and *C. carpio*, survival in populations fed decapsulated cysts or *Artemia* nauplii did not differ significantly from each other (Vanhaecke *et al*., 1990; Jähnichen & Kohlmann, 1999). A comparison of these diets with regard to their effect on growth and development of goldfish has not been done.

Feeding decapsulated cysts instead of nauplii allowed the quantity of cysts to be reduced by 25% to 35% after one to two weeks of culturing, respectively (Vanhaecke *et al*., 1990). Additionally, production costs may also be reduced by feeding cysts that have a low hatch-rate as a result of their improper harvesting and processing because their nutritional value is unaffected (Vanhaecke *et al*., 1990). Therefore, the number of cysts required for larval rearing could potentially be reduced.

Mixed live/dry diets in larval rearing

Fish larvae are known to ingest formulated feeds but they often lack the ability to digest and assimilate them (Holt, 1993). As a result, deformities are common in fish fed dry diets. For example, European minnows *Phoxinus phoxinus* exhibited an increase in malformation with as increase in the proportion of the dry food component in a mixed dry/live diet (Kestemont & Stalmans, 1992). Kestemont & Stalmans (1992) hypothesised that this was a result of the inadequate nutritional value of the dry food. Carp larvae, *C. carpio*, also showed a high rate of malformation when fed dry food alone (von Lukowicz 1976). This was reduced by supplementing the diet with *Artemia* nauplii. Bardi *et al*. (1998) found that the addition of brine shrimp to a mixed diet resulted in an increased growth rate in sturgeon larvae, *Acipenser oxyrinchus desotoi*. Thus, dry food in the diet of some fish species reduced growth and development and this was only corrected through supplementation with live food. In some fish species, the best growth and development of larvae was obtained through early rearing with mixed live/dry diets. Kestemont (1995) achieved the best growth rate in intensively reared goldfish by feeding fish a mixed diet of *Artemia* and dry food but it was not known if the fish fed selectively on the *Artemia* component of the diet. A behavioural study into the feeding behaviour of goldfish larvae and juveniles examines this aspect in chapter 7. Rottmann *et al*. (1991) claimed that a feeding program comprising of a combination of live food items and a dry diet would be superior to a dry diet alone because it would: (1) meet more completely the nutritional requirements of the larvae for maximum growth and survival; (2) provide a range of nutrients to ensure consistent growth; (3) provide back-up food supplies in case of production failures; and (4) probably be less traumatic in the transition from live food to fingerling production rations by introducing the fish to an artificial diet early in life.

One of the problems associated with feeding dry diets is the relatively fast deterioration of water quality and tank cleanliness and the rapid proliferation of micro-organisms on uneaten food (Charlon & Bergot, 1984). This may affect the growth and survival of larvae (Appelbaum & Uland 1979; Rottmann *et al*., 1991; Sharma & Chakrabarti, 1999). However, commercial dry diets are more convenient to feed than live food as they do not require the labour and facilities needed for live food production, and they are not susceptible to production failure (Rottmann *et al*., 1991). The problem of water quality degradation due to feed type is not limited entirely to the dry food component of a mixed diet because uneaten *Artemia* may also reduce water quality. *Artemia* nauplii have a limited lifespan in freshwater and those that are not consumed within the first hour after feeding reduce water quality as they decompose.

Cannibalism in larval and juvenile fish

Diet type has been shown to affect size variation in fish, and where heterogeneity in the size distribution of fish occurs, cannibalism is more prevalent. For example, Von Lukowicz (1979) cited in Kestemont (1995) reported that when feeding *Artemia* combined with a dry diet, it decreased cannibalism in carp larvae compared with larvae fed on dry food alone. Kestemont (1995) also found higher incidence of cannibalism due to the large size variation in treatments where fish were fed dry food only. In addition feeding rate had an effect on cannibalism. For example, feeding carp larvae below a maintenance level of 5% (in set conditions) of body mass per day resulted in increased cannibalism due to large size variation amongst siblings (Kestemont & Mélard, 1991).

Cannibalism can be separated into two types, Type I cannibalism and Type II cannibalism. In Type I, the conspecific larvae are ingested tail first while in Type II, the cannibal ingests

the whole fish head first (Hecht and Appelbaum, 1988). It is necessary for the cannibal's mouth-gape to exceed the head depth of the prey for Type II cannibalism to occur and knowledge of mouth-gape development may help to understand cannibalism in larval and juvenile goldfish.

Most studies on early development of fish have been focused on salmonids and the commercially important cyprinids such as carp (Çalta, 2000). There is a lack of information on juvenile growth and morphological development of goldfish larvae. The results of the study will contribute to knowledge on the early larval rearing of goldfish and help understand how their development is influenced by diet.

Aims and objectives

The aim of this study was to determine the effect of three diets, *Artemia* nauplii, a mixed live/dry diet, or decapsulated cysts, on growth in larval goldfish during the first ten days of feeding. The research objectives were to:

- Test the effect of three different diets, i.e., instar I *Artemia* nauplii, a mixed live/dry diet, and decapsulated *Artemia* cysts, on growth, survival and development of goldfish;
- describe the morphological development of goldfish as a function of diet type; and
- determine if diet type affects cannibalism in order to find potential causal factors for cannibalism.

Materials and methods

Six hours after hatching, larval goldfish were transferred into the experimental aquarium system described in Chapter 2, at a stocking density of 12 fish /L (156 fish per tank). The tanks were randomly assigned to 3 treatments with two replicates for each treatment. The 800-L recirculating system comprised 36 glass tanks, a trickle filter, and a submerged biological filter. Temperature was maintained at 23 ± 1.5˚C by a central heater-thermostat unit in the pump sump of the system. Photoperiod was maintained at 12L:12D by L18 W/72 Biolux tubes (Osram). Aeration was supplied to the pump sump and oxygenated water delivered to each tank via two 5-mm diameter pipes at a total flow rate of 0.6 L/tank/min.

Two days after hatching, the fish were fed either instar I *Artemia* nauplii (93 nauplii per fish per day), rehydrated and decapsulated *Artemia* cysts (93 cysts per fish per day), or a mixed diet consisting of a commercially available dry goldfish feed (0.01g per fish per day; see Chapter 2 for proximate analysis of the dry diet and for *Artemia* batch number) and instar I *Artemia* nauplii (93 cysts per fish per day). The number of Artemia or cysts to be fed each day was estimated by taking the average number of prey items counted under a microscope from three 1-ml samples. The required volume in ml of the concentrated prey items was calculated by the method described in Chapter 2, and then pipetted into each tank. Fish were fed five times per day (08:00h, 10:00h, 12:00h, 14:00h, and 16:00h) for 6 days and then three times per day (08:00h, 12:00h, and 16:00h) for another 4 days. Uneaten food was siphoned from the bottom of each tank every morning before first feeding and dead fish were counted and removed.

A random sample of five fish was removed for measurement from each tank each morning before first feeding and replicates were pooled for each treatment. Total length, standard length/notochord length and head depth were measured to two decimal places, prior to the measurement of mouth-gape (see Chapter 2 for a detailed description of the materials and methods). Incidence of cannibalism was noted each day and survival was determined by counting the fish at the end of the study.

Data were tested for normality using the Kolmogorov-Smirnov test and for equality of variance using Lévene's test. Analysis of variance (ANOVA) was used to test for differences between data that were normally distributed and if differences were observed, Tukey's HSD post-hoc test was used to show where the differences occurred. If data were not normally distributed or if there were unequal variances between treatments, the Kruskall-Wallis test was used to compare treatment medians. If the development of total length, standard length, head depth and mouth-gape over time was linear, data for the three diets were compared using analysis of co-variance (ANCOVA). If differences in growth between treatments were identified, pair-wise comparison between all combinations of treatments was used after correcting the p-value at which the null hypothesis was rejected using the Bonferroni adjustment. The coefficient of variation (CV) is a measure of relative dispersion and was used to compare variation on a particular DAH. CV is defined as $Cv = \frac{\sigma}{\bar{\chi}}.100$ where σ is the standard deviation and $\bar{\chi}$ is the sample's arithmetic mean.

Ratio comparisons of standard length to total length, mouth-gape to total length and mouth-gape to head depth were then plotted to compare all fish of the same age, to test if relative mouth-gape was related to age or size of the fish. The time when metamorphosis occurred was estimated to the nearest DAH from the graphs plotted. From each treatment, the two

highest measurements for mouth-gape were plotted against the two lowest head depths in order to predict the probability that Type II cannibalism might occur. The probability for the head depth of the smallest fish being smaller than the mouth-gape of the largest fish in a treatment was calculated using a z-test. The z statistic was calculated using the following equation:

$$z = \frac{x - \bar{x}}{\delta}$$

where x is the mouth-gape size required to fit the smallest head depth into it and $\bar{x}$ is the arithmetic mean mouth-gape size of the sample. δ is the sample standard deviation for mouth-gape. Then, the probability of finding fish with this mouth-gape was taken from the normal distribution table using the calculated z value.

Results

Diet affected the development of standard length (notochord length), total length, head depth, and mouth-gape (Figures 4.1.1 to 4.1.4) and the respective growth models are shown in table 4.1.

Development of standard length

At 7 DAH, standard length differed significantly ($F_{2;29} = 4.31$; $p \leq 0.05$) between diet types. At this time, larvae fed decapsulated cysts had significantly smaller ($p \leq 0.0001$) notochord lengths than those fed the mixed diet (Figure 4.1.1). Larvae fed *Artemia* nauplii were similar in size on 7 DAH ($p \geq 0.05$) to fish fed either the mixed diet or decapsulated cysts. From 8 DAH to 12 DAH, larvae fed *Artemia* had shorter mean standard lengths than those fed either cysts or the mixed diet. By 12 DAH, diet had exerted a significant effect on standard length ($F_{2;29} = 1.87$; $p \leq 0.0001$). Standard

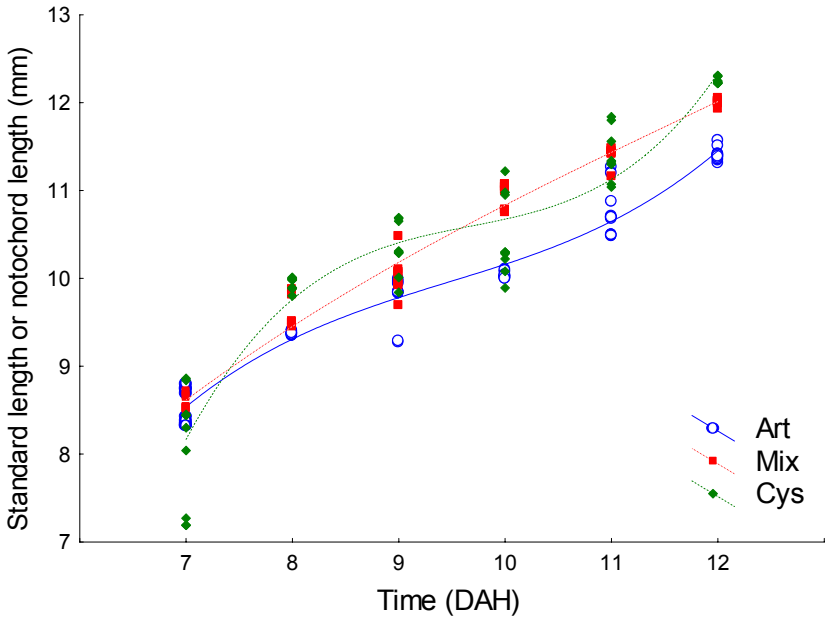


Figure 4.1.1: Increase in standard length (notochord length in pre-flexion larvae) over time (DAH refers to days after hatching) with three diets: Art (instar I *Artemia* nauplii), Mix (mixed diet of *Artemia* nauplii and a dry diet), and Cys (decapsulated *Artemia* cysts).

length was significantly longer ($p \leq 0.0001$) in larvae fed decapsulated *Artemia* cysts (12.25 ± 0.04 mm) than in those fed the mixed diet (12.01 ± 0.03 mm). Larvae fed on instar 1 *Artemia* nauplii had significantly ($p \leq 0.0001$) smaller standard lengths (11.41 ± 0.07 mm) than the larvae fed either the mixed diet or decapsulated cysts. The ranking of mean standard length values on 12 DAH was: cysts > mixed diet > *Artemia* ($p \leq 0.001$).

Development of total length

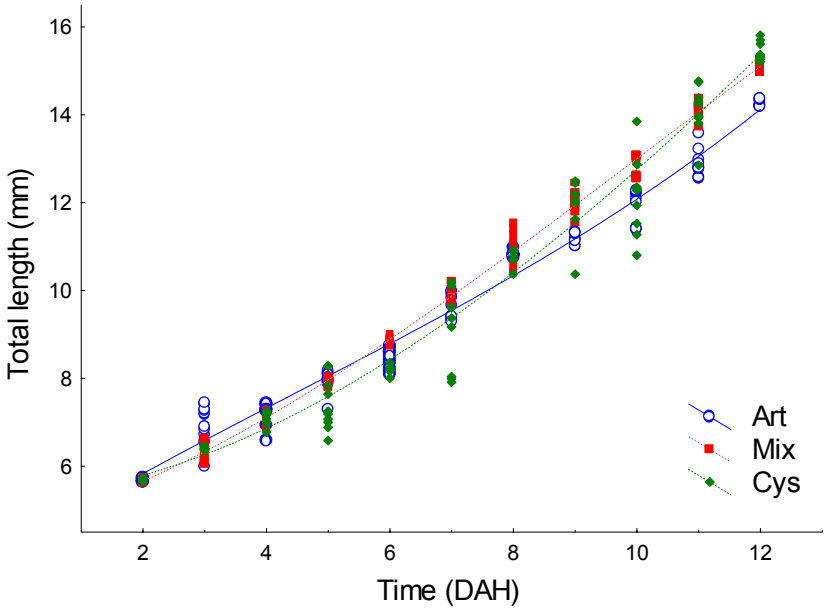


Figure 4.1.2: Increase in total length over time (DAH refers to days after hatching) with three diets: Art (instar I *Artemia* nauplii), Mix (mixed diet of *Artemia* nauplii and a dry diet), and Cys (decapsulated *Artemia* cysts).

Total length of larvae measured at 2 DAH was similar for all treatments ($p \geq 0.05$). At 12 DAH (Figure 4.1.2) mean total length was significantly affected by diet type ($F_{2;29} = 188.6$; $p \leq 0.0001$). Larvae fed decapsulated *Artemia* cysts (15.42 ± 0.21 mm)

had significantly longer total lengths ($p \leq 0.0001$) than those fed the mixed diet (15.13 ± 0.09 mm). Larvae fed on instar 1 *Artemia* nauplii had significantly ($p \leq 0.001$) smaller total lengths (14.26 ± 0.08 mm) than the larvae fed either the mixed diet or decapsulated cysts. The ranking of mean total length values on 12 DAH was: cysts > mixed diet > *Artemia* ($p \leq 0.001$).

Development of head depth

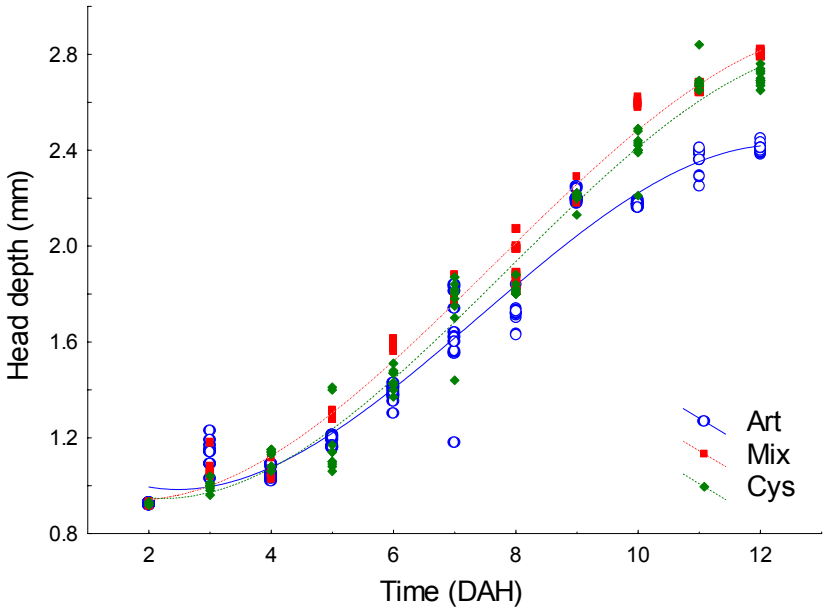


Figure 4.1.3: Increase in head depth over time (DAH refers to days after hatching) with three diets: Art (instar I *Artemia* nauplii), Mix (mixed diet of *Artemia* nauplii and a dry diet), and Cys (decapsulated *Artemia* cysts).

At 2 DAH, the head depths of fish fed *Artemia*, decapsulated cysts or the mixed diet were similar for all treatments ($p \geq 0.05$). At 12 DAH the effect of diet type on head depth was significant ($F_{2;29} = 750.1$; $p \leq 0.001$) with the head depth of those larvae fed decapsulated cysts (2.70 ± 0.03 mm) being significantly smaller ($p \leq 0.001$) than larvae fed the mixed diet (2.80 ± 0.01 mm). Larvae fed *Artemia* nauplii had

significantly smaller ($p \leq 0.001$) head depths (2.41 ± 0.02mm) than those fed either cysts or the mixed diet. Hence, the ranking from largest to smallest head depth at 12 DAH was: mixed diet > cysts > *Artemia* ($p \leq 0.001$).

Development of mouth-gape

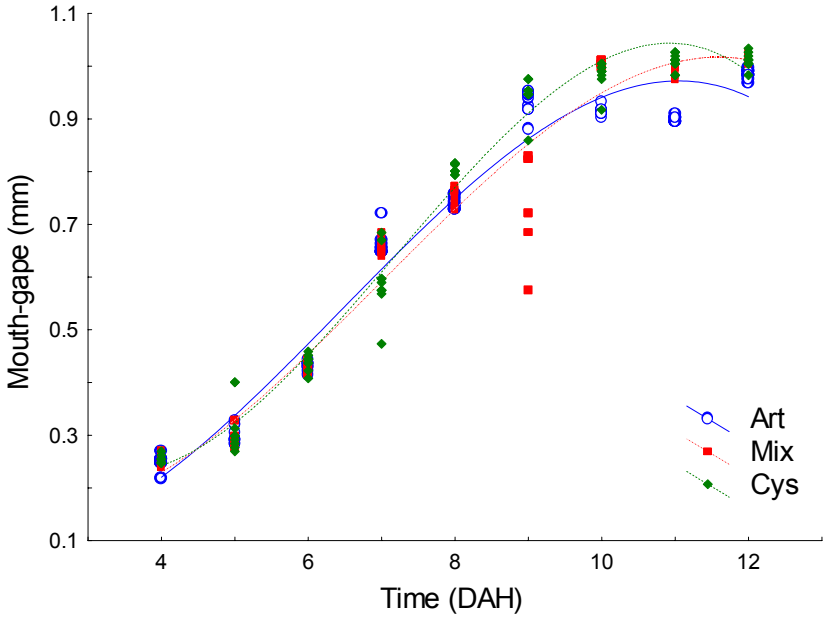


Figure 4.1.4: Increase in mouth-gape over time (DAH refers to days after hatching) with three diets: Art (instar I *Artemia* nauplii), Mix (mixed diet of *Artemia* nauplii and a dry diet), and Cys (decapsulated *Artemia* cysts).

Mouth-gape was first measured on 4 DAH and no significant difference between treatments was found ($F_{2;29} = 0.11$; $p \geq 0.05$). At 12 DAH the effect of diet type on mouth-gape was significant ($F_{2;29} = 24.8$; $p \leq 0.0001$), where the mean mouth-gape of goldfish larvae fed on the mixed diet (1.013 ± 0.008 mm) did not differ significantly ($p \geq 0.05$) from those fed cysts (1.011 ± 0.014 mm) but both were significantly larger

($p \leq 0.001$) than those fed *Artemia* nauplii (0.984 ± 0.008 mm). Ranking of average mouth-gape values at 12 DAH was: cysts = mixed > *Artemia*.

The coefficient of variation (CV) for all measurements was highest in fish fed decapsulated cysts with the exception of standard length but values were all below 1.5% of the mean (Figure 4.2).

Table 4.1: Growth models for standard length (notochord length) (**SL**), total length (**TL**), head depth (**HD**), and mouth-gape (**MG**), in larvae fed either *Artemia* nauplii, a mixed diet of *Artemia* nauplii and dry food, and decapsulated *Artemia* cysts. n is the number of goldfish sampled. x is the time in days.

Diet	Measurement	Growth Model	n
Artemia nauplii	SL	$y = -22.3+9.47x -0.96x^2+ 0.03x^3$	60
Mixed diet	SL	$y = -2.6+2.54x -0.17x^2+0.004x^3$	60
Artemia cysts	SL	$y = -76.9 +26.65x -2.73x^2 +0.09x^3$	60
Artemia nauplii	TL	$y = 4.20 +0.87x -0.03x^2 +0.002x^3$	110
Mixed diet	TL	$y = 4.67 +0.36x +0.07x^2 –0.002x^3$	110
Artemia cysts	TL	$y = 5.29 +0.10x +0.08x^2 –0.002x^3$	110
Artemia nauplii	HD	$y = 1.30 -0.28x +0.07x^2 –0.003x^3$	110
Mixed diet	HD	$y = 1.09 -0.18x +0.06x^2 –0.003x^3$	110
Artemia cysts	HD	$y = 1.20 -0.24x +0.06x^2 –0.003x^3$	110
Artemia nauplii	MG	$y = 0.21 -0.13x +0.04x^2 –0.002x^3$	90
Mixed diet	MG	$y = 0.35 -0.18x +0.05x^2 –0.002x^3$	90
Artemia cysts	MG	$y = 0.88 -0.43x +0.08x^2 -0.004x^3$	90

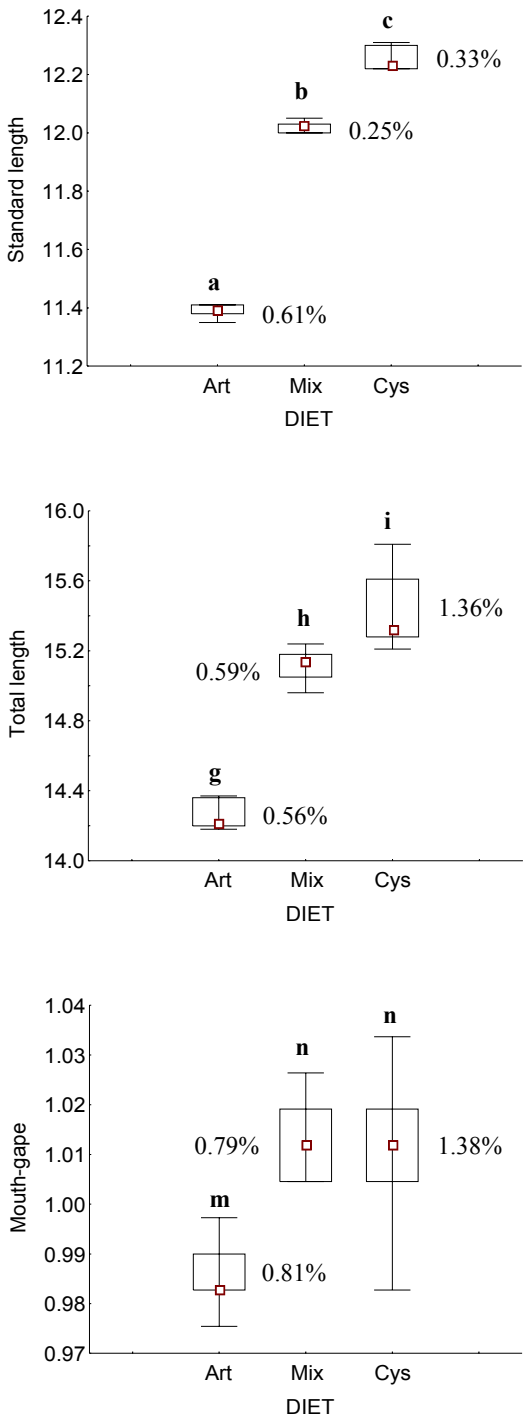


Figure 4.2: Box and whisker plots of the mean values and min-max values 14 DAH (days after hatching) for standard length (notochord length), total length, and mouth-gape with three diets: Instar 1 *Artemia* nauplii (Art), a mixed diet (Mix), and a diet of decapsulated *Artemia* cysts (Cys). The box-section of each plot represents 75% of the data for each treatment whiskers represent the min and max values. The coefficient of variation (%) is displayed next to each plot. Different letters (a,b,c; g,h,i; m,n,o;) denote significant difference ($p \leq 0.05$) within each variable.

Ratios of total length to standard length, mouth-gape to total length and mouth-gape to head depth for larval goldfish fed different diets

Total length: standard (notochord) length

The ratio of total length to standard length (notochord length) was significantly affected by diet ($F_{2;174} = 6.65$; $p \leq 0.05$). This ratio increased at the same rate in larvae fed the mixed diet and *Artemia* (Figure 4.3) but increased fastest in fish fed cysts. The ratio of TL: SL differed significantly between treatments at 7 DAH ($F_{2;29} = 9.8$; $p \leq 0.001$) and at 12 DAH ($F_{2;29} = 3.6$; $p \leq 0.05$). At 12 DAH there was greater variation in the ratio of total length to standard (or notochord) length in fish fed cysts (CV = 1.1%) than in those fed *Artemia* nauplii (CV = 0.5%) and the mixed diet (CV = 0.4%) (Figure 4.3). At 12 DAH the ranking of ratio of total length to standard length was as follows: mixed diet> cysts> *Artemia.*

Mouth-gape: total length

Diet had a significant effect on the mouth-gape to total length ratio by 4 DAH ($F_{2;29} = 4.09$; $p \leq 0.05$). This ratio reached a peak at 9 DAH (Figure 4.4) when mouth-gape was 8.3% of total length in fish fed *Artemia*, 7.9% for the mixed diet, and 8.4% for the diet of decapsulated cysts. At this time, fish fed the mixed diet had a significantly smaller mouth-gape to total length ratio ($F_{2;29} = 10.1$; $p \leq 0.001$) than those fed cysts or *Artemia*. The ratio of mouth-gape to total length differed significantly between diets 12 DAH ($F_{2;29} = 62.4$; $p \leq 0.001$). Mouth-gape to total length ratio was ranked as follows: *Artemia*> mixed diet> cysts.

Mouth-gape: head depth

The ratio of mouth-gape to head depth was significantly affected by diet by 4 DAH ($F_{2;29} = 4.51$; $p \leq 0.05$) with fish fed cysts having a significantly smaller ratio than fish fed either *Artemia* or the mixed diet. The ratio of mouth-gape to head depth (Figure 4.5) reached a peak 9 DAH in fish fed either *Artemia* nauplii or cysts while this peak was reached 10 DAH in fish fed the mixed diet. However, the ratio of mouth-gape to head depth decreased more rapidly in larvae fed cysts, and at 12 DAH was similar to those fish fed the mixed diet ($p \geq 0.05$). At 12 DAH, fish fed on *Artemia* nauplii had significantly higher mouth-gape to head depth ratios ($p \leq 0.05$) than those fed cysts or the mixed diet. Ranking the ratio of mouth-gape to head depth from largest to smallest at 12 DAH gave the following: *Artemia* > cysts = mixed diet.

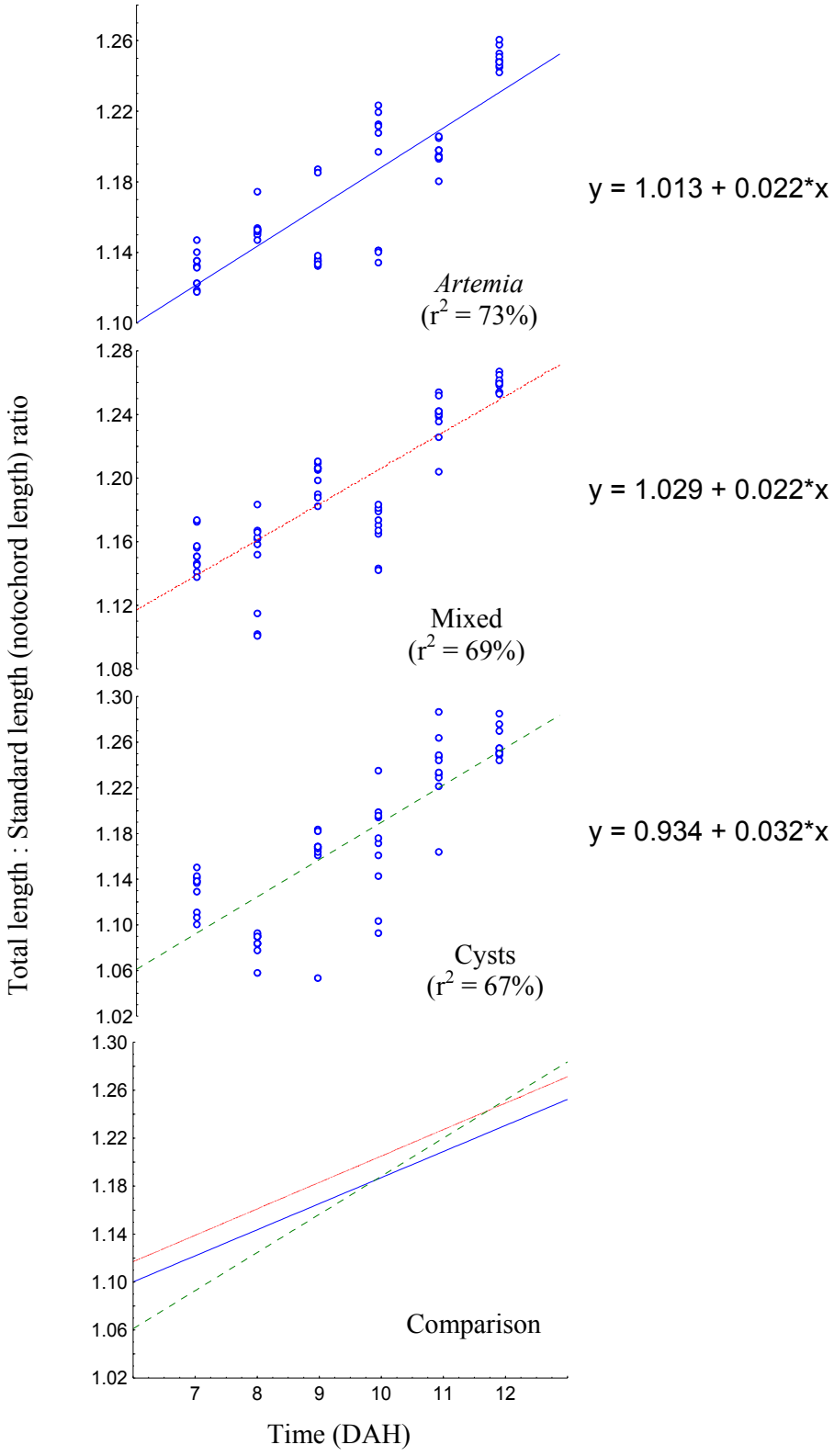


Figure 4.3: Change in the ratio of total length to standard length (notochord length) from 7 DAH (days after hatching) to 12 DAH for fish fed on *Artemia*, mixed diet or cysts. (n = 60 for each treatment). Comparison refers to the superimposed plots of the fitted lines from all three treatments. x = time (days).

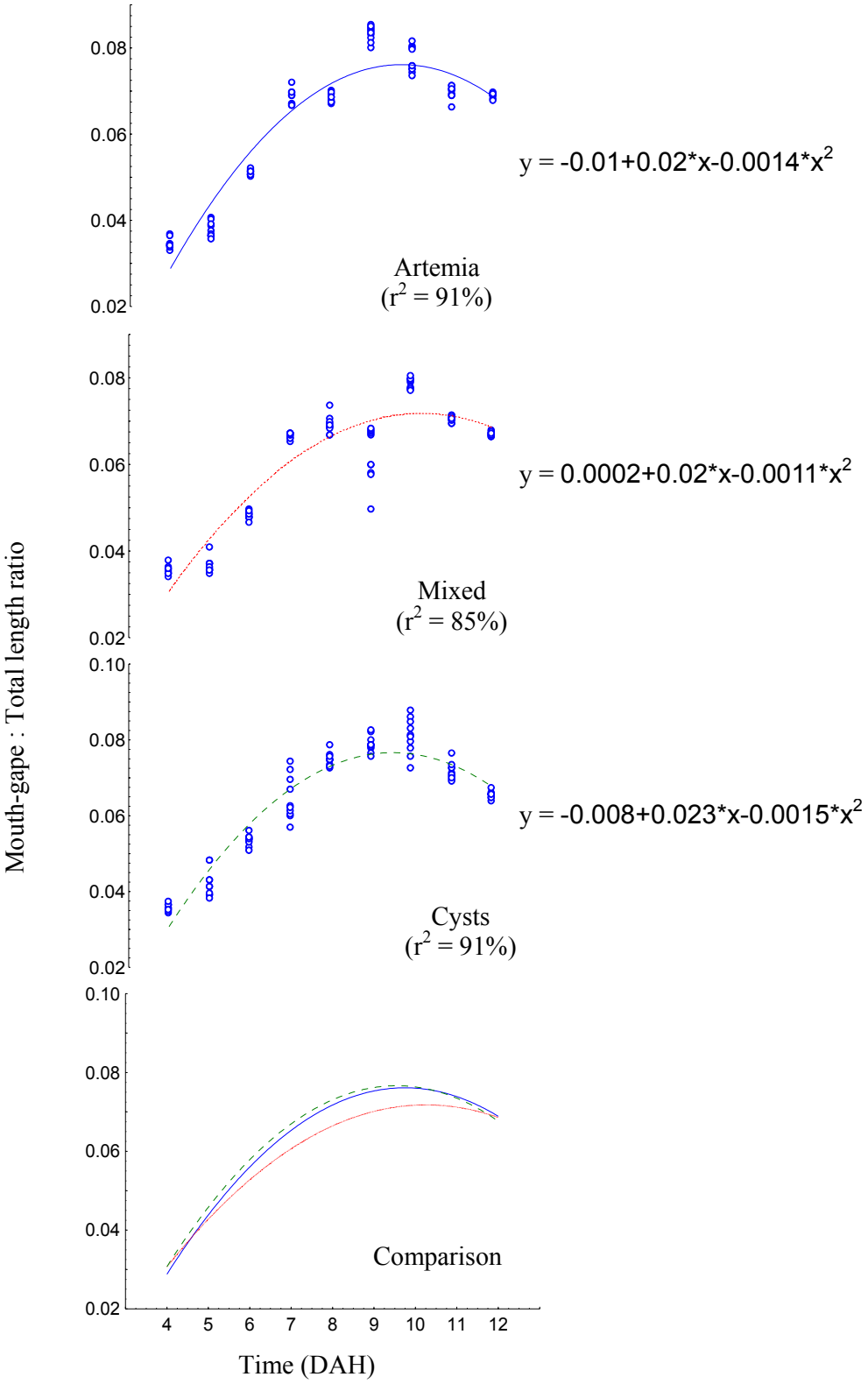


Figure 4.4: Change in the ratio of mouth-gape to total length from 4 DAH (days after hatching) to 12 DAH for fish fed on *Artemia*, mixed diet or cysts. (n = 90 for all treatments). Comparison refers to the superimposed plots of the fitted lines from all three treatments. x = time (days).

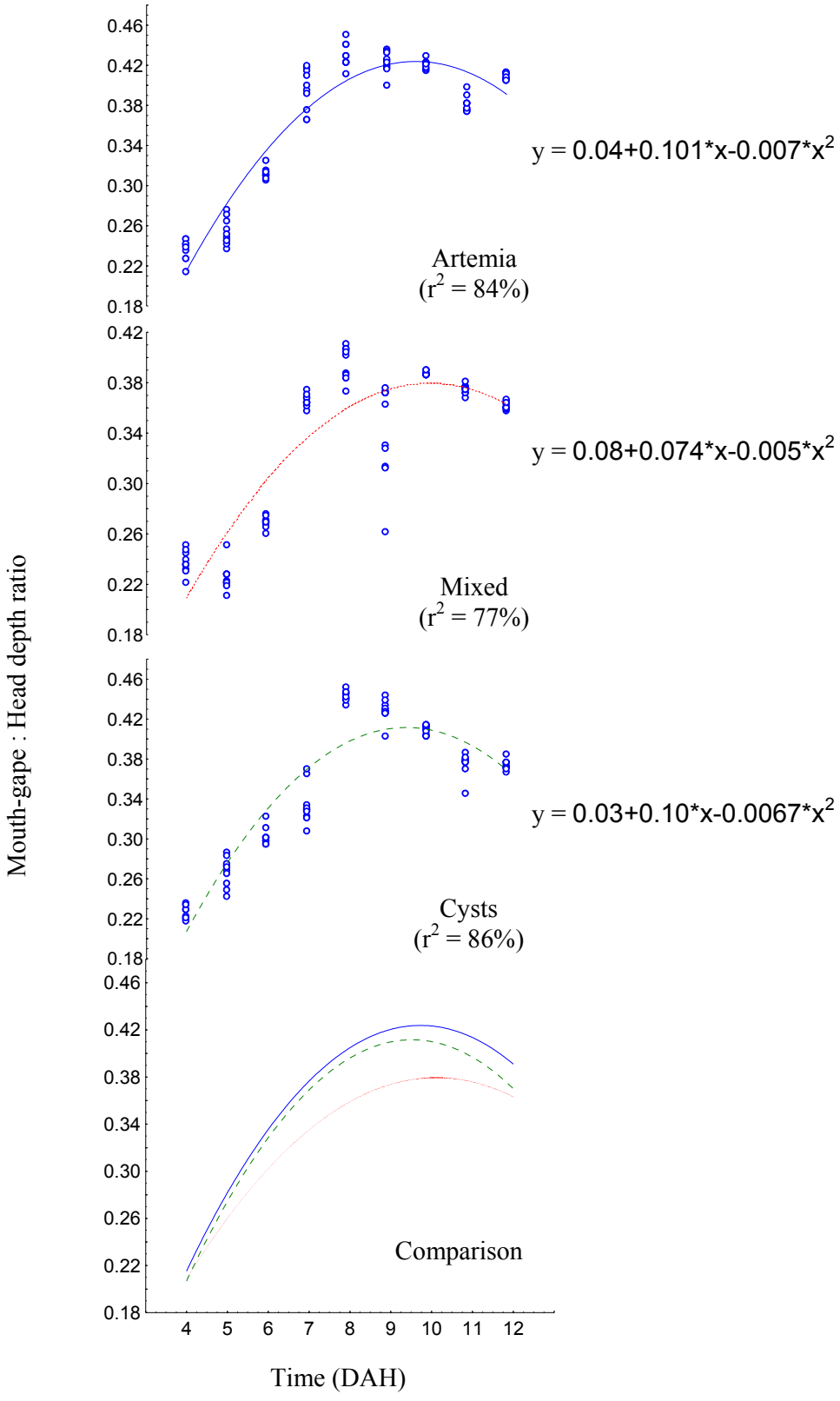


Figure 4.5 Change in the ratio of mouth-gape to head depth over time from 4 DAH (days after hatching) to 12 DAH for fish fed on *Artemia*, mixed diet or cysts. (n = 90 for all treatments). Comparison refers to the superimposed plots of the fitted lines from all three treatments. x = time (days).

In both the *Artemia* nauplii diet and the mixed diet treatment, Type I cannibalism was observed at 5 DAH although it is not known if the fish were scavenged or attacked by their siblings. At 8 DAH, the first incidence of possible Type II cannibalism was observed in fish fed on a diet of *Artemia* nauplii although it is not known if the fish had been scavenged or preyed on. The distributions of mouth-gape and head depth did not overlap. Figure 4.6 shows that the fitted lines for mouth-gape and head depth were diverging with time. The two largest mouth-gapes and the two smallest head depths were compared daily and the 95% confidence bands did not overlap.

Survival at 12 DAH was estimated at 98%, 95%, and 94% for fish fed cysts, the mixed diet, and *Artemia* nauplii, respectively.

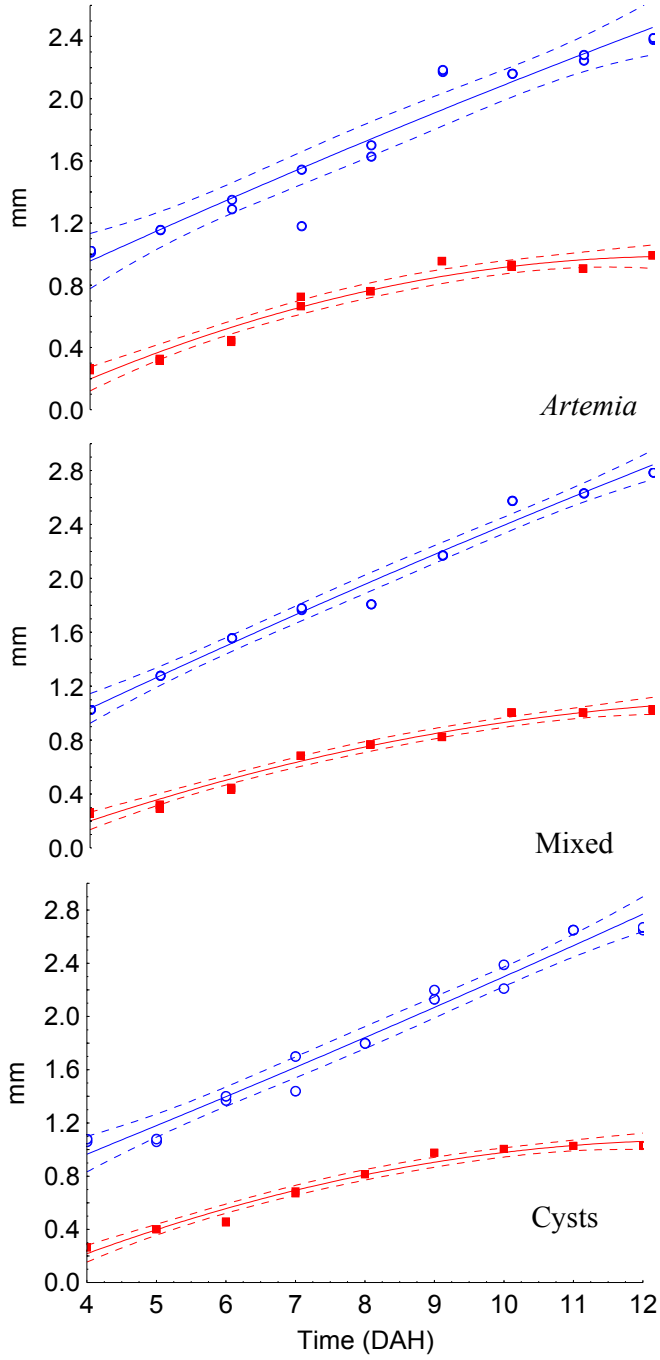


Figure 4.6: Two smallest head depths vs. the two largest mouth-gapes over time with three diets; instar 1 *Artemia* nauplii (*Artemia*), a mixed diet of *Artemia* nauplii and a dry commercial goldfish feed (mixed), and a diet of decapsulated *Artemia* cysts (Cysts). The dashed lines for each plot indicate 95% confidence intervals. DAH refers to days after hatching. x = time (days).

Discussion

Plasticity in morphological development of goldfish larvae during early rearing

Diet modified the morphological development of goldfish larvae. This indicates some degree of phenotypic plasticity in the early development of goldfish. Phenotypic plasticity is defined as the occurrence of morphological variations in response to environmental conditions (West-Eberhard, 1989) such as prey type (Hegrenes, 2001) and nutritional quality of the diet (Wimberger, 1993). Meyer (1987) found that feeding the cichlid, *Cichlasoma managuense, Artemia* or a nematode /flake diet, resulted in distinctly different head shapes after eight months. The results presented in this chapter indicate that goldfish larvae exhibited phenotypic plasticity within the first two weeks of rearing.

The effect of diet on the development of mouth-gape has been described for cyprinid species. For example, Nagelkerke (1997) described the phenotypic plasticity in a *Barbus* species in Lake Tana where 14 different morphotypes resulted from fish feeding on different prey items as well as choice of ecological niche (Nagelkerke, 1997). Thus, by feeding fish larvae on a specific diet, morphological characteristics, such as mouth gape can be modified. Reduced mouth gape has been shown to reduce cannibalism (Dabrowski & Bardega, 1984).

Size variation is a cause of cannibalism in African catfish, *C. gariepinus* (Hecht & Pienaar, 1993). Despite having the largest variations in total length and mouth gape, no cannibalism was observed in cyst-fed goldfish. Conversely, higher rates of cannibalism were recorded in fish fed *Artemia* nauplii or a mixed diet, despite a smaller size variation in their populations. Further support for the theory that it is possible to modify larval and juvenile

morphology though diet selection is provided by Hegrenes (2001), who induced morphological variation in head depth and mouth-gape in the orange-spotted sunfish *Lepomis humilis* by feeding them *Artemia* nauplii, mosquito larvae or mealworms.

There are many examples of diet-induced polymorphism in fish. Schluter (1993, 1995) studied sticklebacks, *Gasterosteus aculeatus*, and found that pelagic planktivorous sticklebacks had more gill rakers than benthic foragers. The author does not mention if the sticklebacks occupying different niches were siblings but the findings indicated the occurrence of phenotypic plasticity. Magnusson & Ferguson (1987) reported four different polymorphisms in the Arctic charr *Salvelinus alpinus* which resulted from different foraging strategies.

The functional significance of the change in body shape is difficult to interpret but it may be inferred from examination of a broad range of fish shapes (Hegrenes, 2001). Benthic feeders are more sedentary and generally have body shapes that are more deep-bodied and less fusiform relative to planktivores or pelagic fish that forage while swimming through open water (Hegrenes, 2001). It is also possible that morphological differences were due to differing physical mechanisms required for ingestion. Small, moving prey items require repeated suction feeding while floating dry food requires a different capture strategy. Such different strategies may lead to different rates of development of the head. In this study, cysts drifted to the bottom of the culture tanks and did not need to be hunted compared with the need to chase after erratically swimming *Artemia* nauplii. This could lead to a conservation of energy, and together with the higher nutritional value of *Artemia* cysts, it could have resulted in the larger total length of larvae fed cysts.

Figures 4.7 and 4.8 provide a ranking of the various changes in development that occurred during the first 12 DAH using Artemia as the reference diet. Fish fed cysts grew faster than those fed nauplii and the mixed diet. It is thus suggested that *Artemia* cysts are more suitable for rearing larval goldfish than nauplii under these conditions.

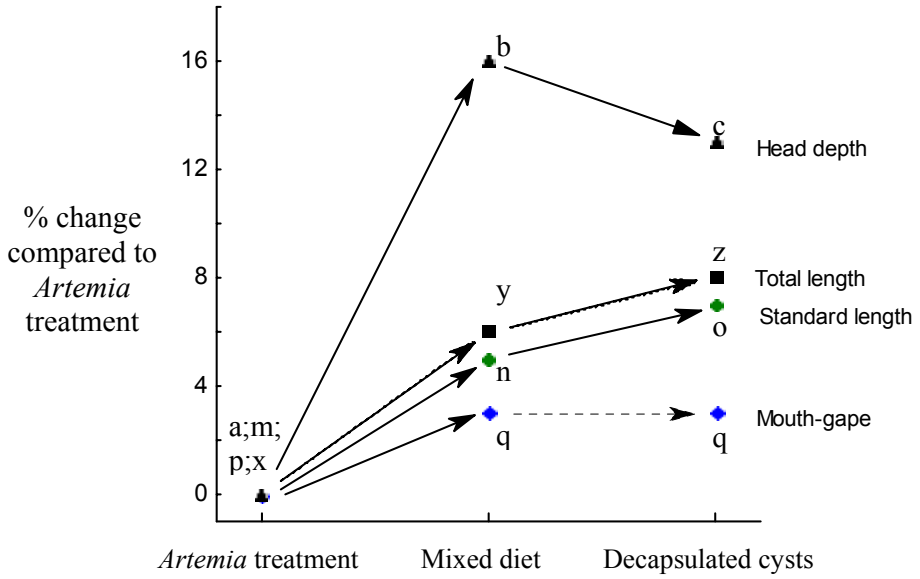


Figure 4.7 Percentage change in growth of various morphological characteristics of fish fed the mixed diet and cysts compared to fish fed *Artemia* nauplii. Different letters within each group (a-b-c; m-n-o; p-q-r; x-y-z) or solid lines denote significant difference ($p \leq 0.01$) for each variable.

The difference in the total length to standard length ratio for fish fed the different diets was small (one to two percent) which indicates a very low variability in this measurement. Thus, diet type affected the development of the head and mouth more than standard length and total length. The sample size in this study was comparable to that used in similar studies on cod larvae (Puvanendran, 1999).

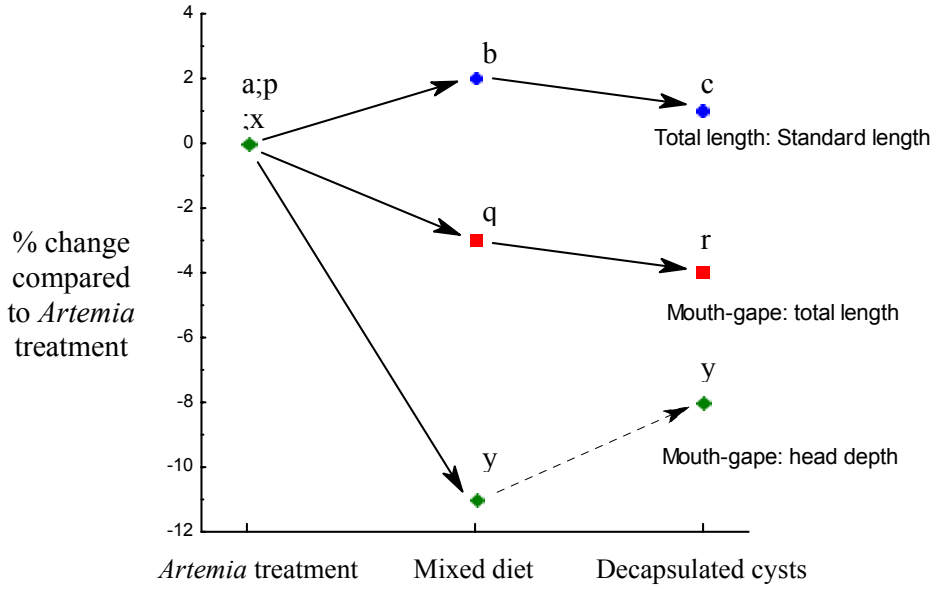


Figure 4.8 Percentage difference in morphometric ratios for fish fed cysts or the mixed diet compared with fish fed *Artemia* nauplii. Different letters within each group (a-b-c; p-q-r; x-y-z) or solid lines denote significant differences ($p \leq 0.01$).

Plasticity in morphological structures has been attributed to changes in growth rate or retarded development (Meyer, 1987), diet quality (Wimberger, 1993), and to adaptive remodelling resulting from differing demands placed on the musculoskeletal system (Wimberger, 1992). Osse *et al.* (1997) hypothesised that the differences in relative sizes of body parts and organs between pre-metamorphic larvae and post-metamorphic juveniles were due to a necessity for setting priorities during early larval growth. Adaptive remodelling may be necessary for fish larvae to complete the development of functional body parts in order to escape predation and obtain external food and by doing so, increase their chance for survival. Results of this study support this hypothesis as there was a change in growth priority from mouth-gape to total length from 9 DAH for fish fed *Artemia* nauplii or cysts, and by 10 DAH in fish fed the mixed diet. This increase in body size in relation to mouth gape coincided with the completion of metamorphosis in goldfish.

Goldfish larvae completed metamorphosis at 10 mm to 10.5 mm standard length at 9 or 10 DAH depending on diet. This was similar to findings by Mills *et al*. (1996) who found that after 11 days of feeding with *Artemia*, all larval goldfish had metamorphosised, while those fed on dry diets had not. Fish were fed more food in this study than they were in Mills *et al*.'s (1996) study. It is hypothesized that this increase in feeding level resulted in faster growth and earlier completion of metamorphosis.

Low feeding levels have been shown to delay the completion of metamorphosis in striped bass *Morone saxatilis* (Eldridge *et al*., 1981) and herring *Clupea harengus* (Werner and Blaxter, 1980). In this study, the dry diet component of the mixed diet delayed the completion of metamorphosis by approximately one day. Since it has been shown that the completion of metamorphosis is more accurately estimated by the size rather than the age of the fish (Fukuhara, 1991), goldfish measuring more than 10.5 mm in total length could be regarded as having completed metamorphosis.

This research demonstrated morphological variation in goldfish larvae in response to diet alone. However, further research is necessary to determine whether the changes in development were a consequence of nutritional constraints or functional adaptation to prey type.

Practical applications

According to Shirota (1970) growth of first feeding larvae is affected by the relationship between mouth gape and larval length. Cunha and Planas (1999) support Shirota's hypotheses by suggesting that larger mouth-gape to standard length ratios resulted in faster growth in turbot larvae, *Scophthalmus maximus* L. They attributed this to the larvae being

able to ingest larger prey at each strike and so maximising the energetic return for each prey capture effort. This forms the basis for the "optimal foraging theory" which states that animals forage in such a way as to maximise the benefit to cost ratio. The optimal foraging theory could explain why fish fed *Artemia* nauplii had the highest mouth-gape to standard length ratio yet were significantly smaller than those fed either cysts or the mixed diet. The energy expended chasing and capturing *Artemia* nauplii may have been greater than the energy requirement for finding cysts, which resulted in the development of a relatively large mouth-gape. In Chapter 7, preference for cysts or nauplii is quantified to assess prey preference.

The relatively larger mouth-gape of fish fed *Artemia* could explain why Type I cannibalism was observed on two occasions at 5 DAH while no cannibalism was observed in fish fed *Artemia* cysts. Type I cannibalism was also observed in fish fed the mixed diet but it is not known whether the fish were actually captured or scavenged from the bottom of the tank. In Chapter 7, results from behavioural observations on the interactions between fish areused to quantify the onset and occurrence of aggressive contacts between siblings.

Bryant & Matty (1981) suggested that cannibalism may be related to low feeding levels. Larvae fed cysts had better survival than those fed either *Artemia* nauplii or the mixed diet. This is similar to findings by Dhert *et al*. (1997) for guppies *P. reticulata*. However, in the larvae of grass carp *C. idella* and common carp *C. carpio*, survival rates for treatments fed decapsulated cysts or *Artemia* nauplii did not differ significantly from one another (Vanhaecke *et al*., 1990; Jähnichen & Kohlmann, 1999). In this experiment, fish were fed to apparent satiation which may have reduced the incidence of cannibalism.

Mouth-gape and diet selection

Knowing mouth-gape size allows estimation of the maximum diet particle size that can be fed to larval fish. Mouth-gape sets the upper size limit for ingestion of food particles. Therefore, the largest particle size that goldfish larvae can ingest 12 DAH under the conditions of this experiment would be less than 1.011 ± 0.014 mm for larvae fed cysts, 1.013 ± 0.008 mm for larvae fed a mixed diet, and 0.984 ± 0.008 mm for larvae fed *Artemia* nauplii.

Advantages of feeding decapsulated cysts

This study revealed various advantages to feeding cysts. One advantage was improved tank hygiene. The dry food component of the mixed diet caused a rapid build-up of fungus in the tanks. Thus, some food was not available to the larvae as it settled in the fungal layer growing on the tank floor. Deterioration in water quality is a common problem associated with feeding dry diets (Charlon and Bergot, 1984). Decapsulated cysts also sank to the bottom of the culture tanks but there was no problem with fungal growth and the larvae fed on cysts from the bottom of the tanks. The possible benefit of keeping cysts suspended within the water column will be investigated in Chapter 5. *Artemia* nauplii lived long enough in the culture tanks to be preyed on, hence very little fouling from uneaten food occurred. The feeding of decapsulated cysts is the least labour intensive of the three diets tested and cyst-fed fish attained the largest size at 12 DAH. This study has shown that feeding cysts to goldfish larvae is a viable alternative to feeding *Artemia* nauplii. To further compare the effectiveness of decapsulated cysts and *Artemia* as a starter diet for larval goldfish it is necessary to test a tank design that can be used on an experimental and commercial scale (Chapter 5), determine the optimal feeding level for cysts (Chapter 6), and to observe prey preference in larvae and juveniles (Chapter 7).

Chapter 5

Growth and development of larval goldfish fed *Artemia* cysts which either sank to the tank bottom or were kept suspended in the water column

Introduction

Larval diets for commercial aquaculture need to be water stable and accepted, ingested, digested and assimilated at rates comparable to live feeds (Jones *et al*., 1993). The results from chapter 4 indicated that goldfish larvae grew better on a diet of rehydrated and decapsulated *Artemia* cysts than on instar I *Artemia* nauplii or a mixed live/dry diet of *Artemia* nauplii and a commercially available goldfish diet. However, despite their apparent nutritional benefits, decapsulated cysts are often difficult to access by the fish as they may sink to the bottom of the culture tank. For example, decapsulated and rehydrated cysts could not be successfully used for feeding carp larvae because they sank too rapidly and the larvae did not feed off the bottom of the tank (Vanhaecke *et al*., 1990).

Chapter 4 showed that goldfish larvae ingested some cysts from the bottom of the culture tank. It has not been tested if the growth of goldfish larvae could be improved by suspending cysts in the tanks thereby making them more accessible. Re-drying decapsulated cysts has been shown to improve the availability of cysts to carp larvae during the first week of culturing (Verreth & Den Bieman, 1987), as the cysts floated for a period of time before sinking to the bottom.

One potential problem with feeding cysts is shown by results from Chapter 4 whereby size variation was greatest in cyst-fed fish. Thus, it should be tested whether this was due to only a portion of the population being able to feed on decapsulated cysts from the tank bottom. It is hypothesised that by increasing accessibility of cysts, size variation among larvae will decrease and growth will improve. This could be achieved by altering the tank design and water inflow, thus suspending decapsulated *Artemia* cysts in the water column. For the purpose of this study, tanks had v-shaped bottoms and an up-welling water inflow to keep cysts floating.

The benefit of feeding either *Artemia* nauplii or cysts to larvae of marine and freshwater fish is reported in the literature. For example, Bardi *et al*. (1998) showed that the addition of brine shrimp, and especially of decapsulated cysts, elicited a strong swimming, search, and feeding stimulus in sturgeon larvae, *Acipenser oxyrinchus desotoi*. Furthermore, Von Lukowicz (1979), cited in Kestemont (1995), found that by feeding a continuous supply of live food such as *Artemia* to common carp larvae, the incidence of cannibalism was significantly lower than for fish fed dry diets, possibly because these fish had a larger size variation (Kestemont, 1995). Large size variation induces cannibalism and agonistic behaviour among fish larvae. Two main factors determining the degree of size variation are food availability and feeding frequency (Hecht & Pienaar, 1993). A wide size distribution in gilthead sea bream *Sparus aurata* caused growth suppression in small individuals (Ruzzante, 1994). It can also increase cannibalism in some piscivorous species (Gunnes, 1976). A large size variation has been attributed to the lack of an optimal food particle size and form (Wankowski & Thorpe, 1979) and an inconsistent feeding level (Wickins, 1985; Abi-Ayad & Kestemont, 1994; Goldan *et al*., 1998).

Commercially reared fish are size-graded to reduce problems associated with size variation (Goldan *et al*., 1998). It would be beneficial if manipulating feeding rate and diet type can control size variation and reduce the need for frequent size-grading, an activity which is stressful to fish. Among the more obvious benefits such as a reduction in cannibalism, size grading in some species (e.g. Arctic charr, *Savelinus alpinus*) disrupted the development of social hierarchies and allowed smaller fish to grow better in the absence of dominant fish (Gunnes, 1976; Wallace & Kolbeinshavn, 1988). It is also more convenient to have fish of similar size in the culture tanks for selection of optimal food size. Gunnes (1976) found that size grading Atlantic salmon *Salmo salar* at an early age lead to better growth than sorting at a time when social structure may have developed.

Stocking density has been shown to influence size variation in juvenile turbot *Scophthalmus maximus* (Irwin *et al*., 1999) where increased stocking density reduced growth and survival and increased size variation. However, stocking density did not affect size variation in European sea bass *Dicentrarchus labrax* (Hatziathanasiou *et al*., 2002). In this study, stocking density s reduced daily through sampling. Thus, the effect of reducing the stocking density of goldfish larvae is investigated.

Aims and objectives

The aim of this study was to determine the effect of keeping decapsulated *Artemia* cysts suspended in the water column on growth and survival of larval goldfish. A second experiment aimed to test the effect of suspending cysts in the water column for two months at a constant stocking density. The control groups were fed cysts that sank to the bottom of the tank. The research objectives for this study are to:

- Determine the effect of suspending *Artemia* cysts in the water column on growth and survival of larval goldfish over the first 16 DAH and at 62 DAH;
- determine if suspending *Artemia* cysts in the water column has an effect on the size variation of larval goldfish over the first 16 DAH and at 62 DAH; and
- determine the effect of continuously reducing the population density of larvae on growth and survival.

Materials and methods

Six hours after hatching, larval goldfish were transferred into the 800-L recirculating aquarium system that comprised 36 glass tanks, a trickle filter, and a submerged biological filter as described in Chapter 2. Eight tanks were randomly assigned to treatments with two replicates for each treatment.

Temperature was maintained at 23 ± 1.5˚C by a central heater-thermostat unit in the system sump. Photoperiod was maintained at 12L: 12D by an L18 W/72 Biolux tube (Osram) above each tank. Aeration was supplied to the pump sump and oxygenated water delivered to each tank via two 5-mm diameter pipes at a flow rate of 0.6 L/min for the flat-bottomed tanks and via a single, perforated pipe for tanks with the V-shaped bottom design.

Fish were stocked into each tank at twelve fish per litre. From two days after hatching, the fish were fed decapsulated *Artemia* cysts at 150 cysts /fish /day. The number of prey items was calculated using the method described in Chapter 2. Fish were fed five times per day at 08:00, 10:00, 12:00, 14:00 and 16:00 until eight days after hatching and then three times per day (08:00, 12:00 and 16:00) until the end of the experiment. Each day before first feeding, water flow was turned off to allow solids to settle and any uneaten cysts were siphoned from the tank bottom. Dead fish were removed and counted daily. The treatments, each with a replicate, included: (A) a V-shaped tank bottom with a perforated water inlet pipe along the bottom of the tank to ensure up-welling water circulation in the tank (Figure 2.2; Chapter 2); and (B) a flat-bottomed tank with the water inflow at the surface.

Total length, standard length/notochord length, head length, body length and head depth were measured in millimetres to two decimal places, prior to the measurement of mouth-

gape for each specimen (see chapter 2 for a detailed description of the materials and methods). Tail length was calculated by subtracting standard length from total length. Instances of cannibalism, such as a juvenile swimming with a sibling protruding from its mouth, were noted.

Experiment A: A comparison of V-shaped and flat-bottomed tanks

A random sample of five fish was removed from each tank each morning before first feeding and replicates of each treatment were pooled. At 16 DAH all fish were counted to determine survival.

Experiment B: A comparison of V-shaped and flat-bottomed tanks at 16 DAH and 62 DAH

Fish were fed 150 cysts per fish per day for 62 days. From 16 DAH their diet was supplemented with a dry diet. The dry food (AquaNutro Goldfish Food; WPK, Malmesbury, South Africa) was crushed and sieved to a particle size ranging from 200 μm to 400 μm. The dry diet proximate analysis is described in Chapter 2. A random sample of five fish was removed for measurement from each tank at 16 DAH before first feeding and the replicates were pooled for each treatment. At 62 DAH, ten randomly chosen fish were sampled from each tank and replicates were pooled for each treatment. All fish were counted at 16 DAH and 62 DAH to determine survival for each treatment.

Statistical analysis

Data were tested for normality using the Kolmogorov-Smirnov test and for equality of variance using Lévene's test. If data were not normally distributed or if there were unequal variances between treatments, the Mann-Whitney U-test was used to compare treatment

medians. Development of total length, standard length, tail length, head depth, body depth and mouth-gape over time was plotted for each treatment and if regression lines were linear, data were compared using analysis of co-variance (ANCOVA). ANCOVA was also used to test for any difference between regression models that showed the relationship between body depth and total length. A Student's t-test was used to compare larval characteristics for v-shaped and flat-bottomed treatments at 62 DAH to determine if these treatments differed significantly from each other. The Mann-Whitney U Test was used to describe differences in total length and body depth between experiments A and B at 16 DAH. At 16 DAH, Bartlett's Test (Ott, 1988) was used to compare the variance for total length, standard length, tail length, head depth, body depth and mouth-gape for each treatment.

The coefficient of variation (CV) is a measure of relative dispersion and was used to compare variation in the measured population.

$$Cv = \frac{\sigma}{\bar{\chi}} \bullet 100$$; where σ is the standard deviation, and $\bar{\chi}$ is the mean.

Ratios of standard length to total length, mouth-gape to total length and mouth-gape to head depth for each treatment were compared at 16 and 62 DAH using ANOVA to test if relative mouth-gape was related to age or size of the fish.

Results

Tank design affected the development of total length, standard length (notochord length), tail length, head depth, body depth, and mouth-gape (Figures 5.1 and 5.2).

Development of total length of goldfish larvae and juveniles reared in flat-bottomed tanks and in tanks with a v-shaped bottom

Increase in total length during the first 16 DAH was significantly affected by tank design ($F_{1;275}$ = 7.42; $p \leq 0.01$) (Figure 5.1) with fish reared in tanks with v-shaped bottoms growing faster than those in flat-bottomed tanks. Size variation, expressed as coefficient of variation, increased daily from 2 DAH to 16 DAH up to a maximum of 12 % for fish in flat-bottomed tanks, but varied from 5.5 % to 8 % for fish in v-shaped tanks. The variation in total length of fish from flat-bottomed tanks was relatively high at 9, 11, 15, and 16 DAH when both the longest and shortest fish were from flat-bottomed tanks.

62 DAH, fish reared in v-bottomed tanks had a significantly longer average total length (t = 13.3; $p \leq 0.0001$) than those from flat-bottomed tanks (36.0 ± 1.78 mm and 25.5 ± 3.04 mm, respectively) and the coefficient of variation was lower in v-bottomed tanks (CV = 5 %) than in flat-bottomed tanks (CV = 13 %) (Figure 5.2).

Standard length of goldfish larvae and juveniles reared in tanks with flat or v-shaped bottoms

Development of standard length differed significantly between treatments ($F_{1;175}$ = 8.51; $p \leq 0.005$) during the first 16 DAH. At 16 DAH the coefficient of variation for v- and flat-

bottomed treatments was 5 % and 12 %, respectively (Figure 5.2). At 62 DAH, fish reared in tanks with v-shaped bottoms had a significantly longer mean standard length ($t = 27.7$; $p \leq 0.0001$) than those from flat-bottomed tanks (27.76 ± 1.45 mm and 19.35 ± 2.23 mm, respectively).

Tail length of goldfish larvae and juveniles reared in tanks with flat- or V-shaped bottoms

Tail length increase from 6 DAH to 16 DAH was significantly affected by tank design ($F_{1;175} = 9.98$; $p \leq 0.0001$) and at 16 DAH the coefficient of variation was 17 % and 30 % for v- and flat-bottomed tanks, respectively. At 62 DAH, average tail length was significantly larger ($t = 7.42$; $p \leq 0.0001$) in fish kept in tanks with v-shaped bottoms (8.24 ± 0.4 mm) compared to those from flat-bottomed tanks (6.17 ± 0.18 mm). The coefficient of variation for v- and flat-bottomed tanks was 5 % and 19 %, respectively.

Head depth of goldfish larvae and juveniles reared in tanks with flat- or v-shaped bottoms

Development of head depth from 2 DAH to 16 DAH differed significantly between treatments ($F_{1;275} = 8.96$; $p \leq 0.003$). At 16 DAH, the coefficient of variation for v-shaped and flat-bottomed treatments was 12 % and 19 %, respectively. At 62 DAH, the average head depth of fish reared in tanks with v-shaped tank bottoms (9.63 ± 0.12 mm) was significantly larger ($t = 33.1$; $p \leq 0.0001$) than the average head depth of fish from flat-bottomed tanks (8.39 ± 0.11 mm). The coefficient of variation for both treatments was 1 % (Figure 5.2).

Body depth of goldfish larvae and juveniles reared in tanks with flat- or v-shaped bottoms

Development of body depth from 2 DAH to 16 DAH differed significantly between treatments ($F_{1;275} = 5.14$; $p \leq 0.05$). The coefficient of variation at 16 DAH for the v-shaped and flat-bottomed treatment was 14 % and 21 %, respectively. Average body depth at 16 DAH was 2.0 ± 0.29 mm for v-shaped and 1.85 ± 0.39 mm for flat-bottomed tanks. At 62 DAH, average body depth was significantly larger ($t = 22.56$; $p \leq 0.0001$) in fish from v-shaped tanks (13.48 ± 0.44 mm) than in those from flat-bottomed tanks (9.79 ± 0.58 mm). The coefficient of variation for body depth at 62 DAH was 3 % for the v-shaped treatment and 6 % for the flat-bottomed treatment.

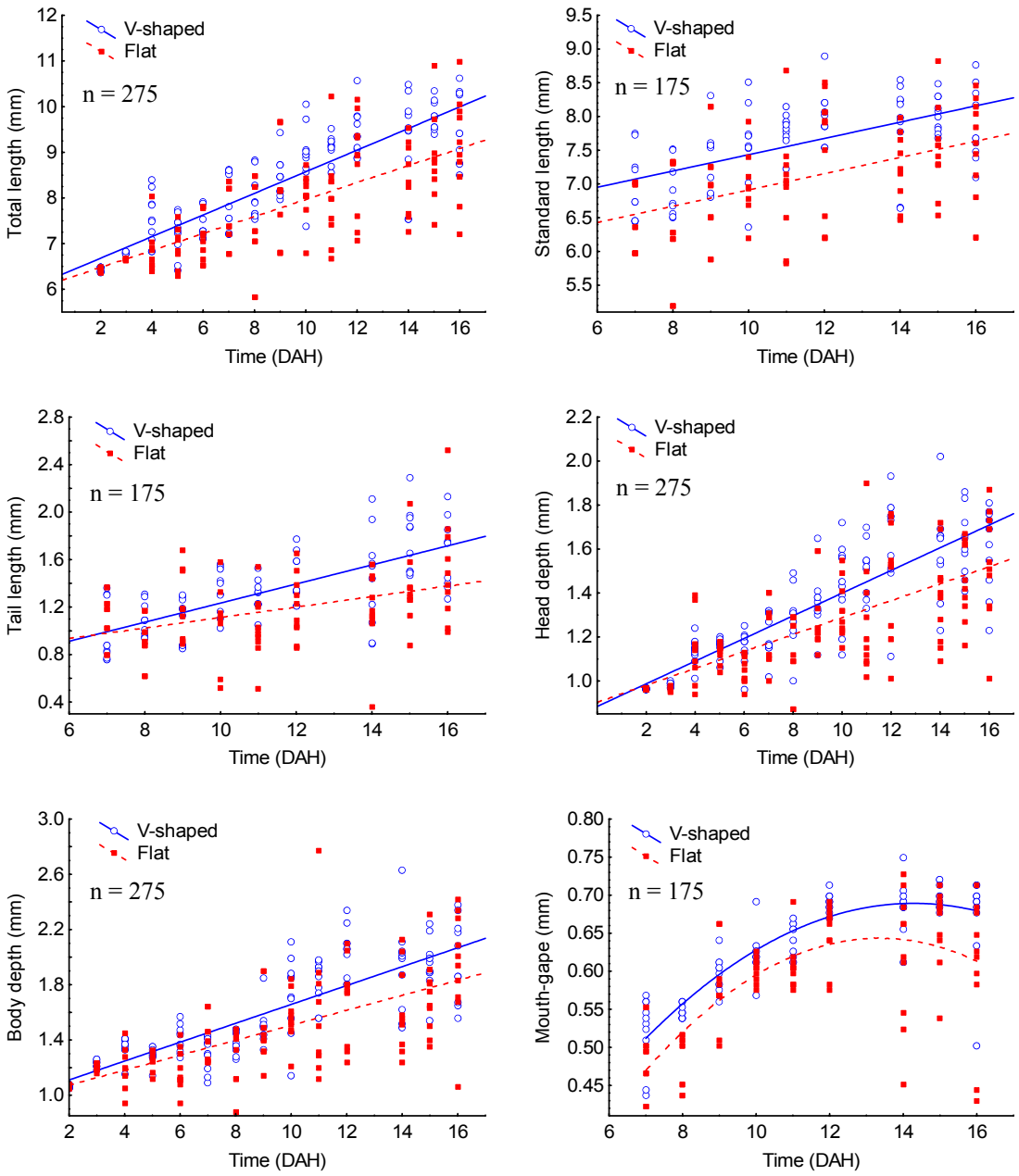


Figure 5.1. Increase in total length, standard length, tail length, head depth, body depth and mouth-gape of goldfish larvae during the first 16 DAH showing differences in morphometric development. Fish were raised either in flat-bottomed tanks (Flat) where decapsulated *Artemia* cysts settled to the tank floor, or in tanks with a v-shaped bottom design (V-shaped) in which cysts were kept suspended in the water column.

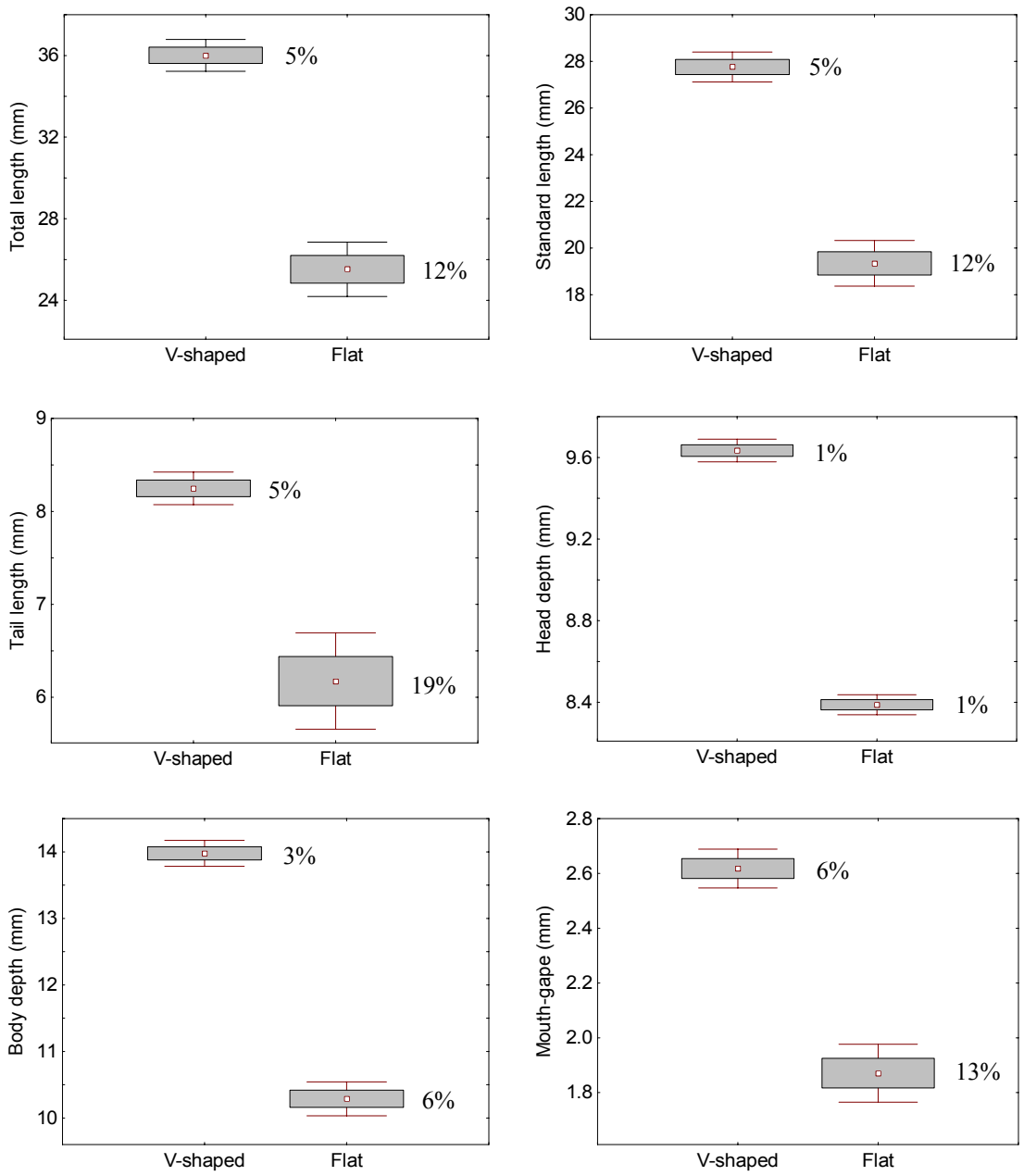


Figure 5.2. Box and whisker plots showing mean values ± standard error at 62 DAH for total length, standard length, tail length, head depth, body depth and, mouth-gape for larval goldfish grown in tanks with either v-shaped bottoms (V-shaped) or tanks with flat bottoms (Flat). The coefficient of variation (%) is displayed next to each plot. All treatments were significantly different from each other ($p \leq 0.0001$). n = 20 for each variable. Whiskers on each plot represent ± 95 % confidence interval.

Mouth-gape development of goldfish larvae and juveniles reared in tanks with v-shaped or flat bottoms

Development of mouth-gape from 2 DAH to 16 DAH is shown in Figure 5.1. The coefficient of variation increased daily until 16 DAH when it was greater in the flat-bottomed treatment (16 %) than in the v-shaped treatment (9 %). The mouth-gape of one fifth of juvenile goldfish from flat-bottomed tanks increased by less than 0.02 mm from 7 DAH to 16 DAH while the average increase in mouth-gape for the remaining fish was 0.14 mm. Average mouth-gape of fish in the v-shaped treatment increased from 0.67 ± 0.06 mm at 16 DAH to 2.62 ± 0.16 mm at 62 DAH. In fish from flat-bottomed tanks there was an increase in mouth gape from 0.60 ± 0.10 mm to 1.87 ± 0.24 mm. At 62 DAH fish from tanks with v-shaped bottoms had a significantly larger average mouth-gape than those from flat-bottomed tanks ($t = 11.49$; $p \leq 0.0001$) with a coefficient of variation of 13 % and 6 % for flat- and v-shaped tanks, respectively.

Summary of trends in morphometric development according to tank design

At 16 DAH, the coefficients of variation for all morphometric variables were higher in fish from flat-bottomed tanks than in those from tanks with a v-shaped bottom. The variation is illustrated by Figure 5.1, which indicates that one or two fish sampled each day from flat-bottomed tanks grew slowly or not at all whereas fish reared in the v-shaped tank design grew relatively well. At 62 DAH, tank design affected the development of juvenile goldfish and those from the v-shaped treatment had a significantly larger total length, standard length, tail length, head depth, body depth, and mouth-gape compared to fish from flat-bottomed tanks ($p \leq 0.0001$). Variation around the mean for the morphometric measurements in fish from flat-

bottomed tanks was highest at 62 DAH with the exception of head depth where the coefficient of variation was 1 % for each treatment.

Table 5.1. Overview of the effect that tanks with v-shaped bottoms (V) and tanks with flat bottoms (F) had on the coefficient of variation (CV) and size ranking of goldfish larvae at 16 DAH (days after hatching) and 62 DAH. "n" represents the sample size for each treatment. Size differences are valid at $p \leq 0.0001$.

Measured parameter	Ranked CV at 16 DAH	Ranked CV at 62 DAH	Average size ranking at 62 DAH (n = 20)
Total length	V < F	V < F	V > F
Standard length	V < F	V < F	V > F
Tail length	V < F	V < F	V > F
Head depth	V < F	V = F	V > F
Body depth	V < F	V < F	V > F
Mouth-gape	V < F	V < F	V > F

Effect of reducing stocking density on total length and body depth

At 16 DAH, the reduced stocking density in experiment A had no effect on total length in either v-bottomed ($Z_{2,19} = 0.60$; $p \geq 0.05$) or flat-bottomed ($Z_{2,19} = 0.08$; $p \geq 0.05$) treatments compared with the constant stocking density in experiment B. Body depth was similar for v-shaped ($Z_{2,19} = 0.57$; $p \geq 0.05$) and flat-bottomed ($Z_{2,19} = 0.23$; $p \geq 0.05$) treatments.

Ratio comparisons

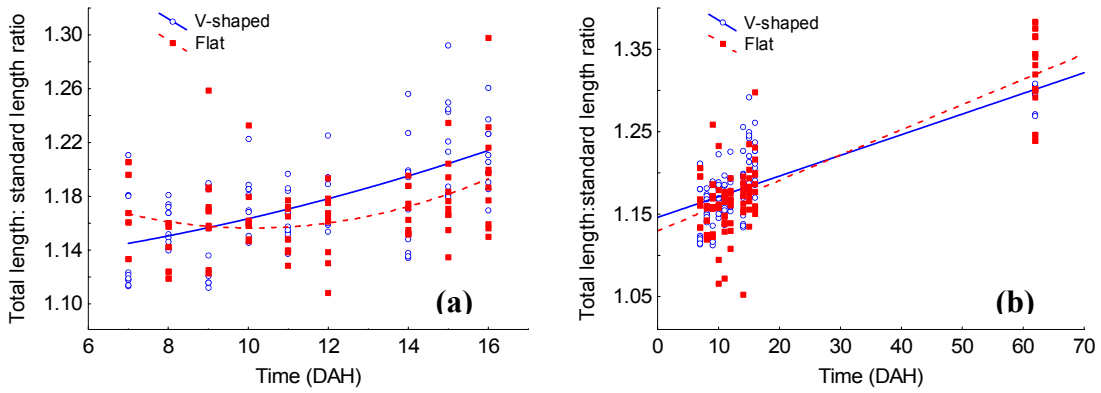


Figure 5.3. Total length to standard length ratio over time for goldfish larvae raised under two different conditions, a v-shaped tank bottom design which kept decapsulated *Artemia* cysts suspended (V-shaped) and a flat bottomed tank design in which cysts settled on the bottom of the tank (Flat). (a) represents ratio development until 16 DAH (n = 175) and (b) represents development until 62 DAH (n = 215).

Total length: standard length ratio

The development of the ratio of total length to standard length was significantly affected by tank design ($F_{1;215} = 5.49$; $p \leq 0.05$) from 7 DAH to 62 DAH (Figure 5.3). At 62 DAH, fish reared in flat-bottomed tanks had a longer average total length in relation to standard length compared to those from tanks with v-shaped bottoms ($t = 1.98$; $p \leq 0.05$). Table 5.2 lists the least square regression models for each ratio comparison.

Mouth-gape to total length ratio

The ratio of mouth-gape to total length reached a maximum on 12 DAH for fish reared in flat-bottomed tanks and on 13 DAH for fish in v-bottomed tanks (Figure 5.4). Mouth-gape in

relation to total length increased from 7 DAH to 62 DAH but this increase did not differ between treatments ($F_{1;215} = 0.02$; $p \geq 0.05$) (Table 5.2).

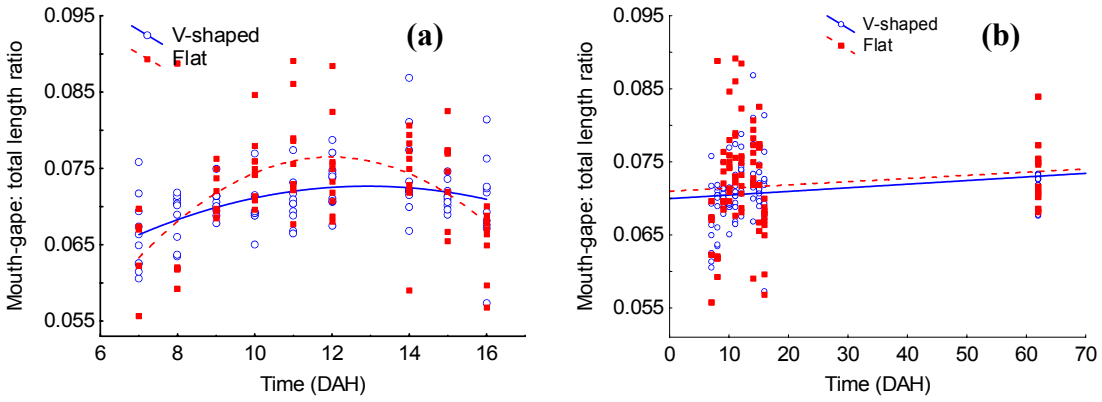


Figure 5.4. Mouth-gape to total length ratio over time for goldfish larvae raised under two different conditions, a v-shaped tank bottom design which kept decapsulated *Artemia* cysts suspended (V-shaped) and a flat bottomed tank design in which cysts settled on the bottom of the tank (Flat). (a) represents ratio development until 16 DAH (n = 175) and (b) represents development until 62 DAH (n = 215)

Mouth-gape to head depth ratio

Mouth-gape to head depth ratio reached a maximum at 12 DAH in fish from flat-bottomed tanks but remained constant until 16 DAH in fish from v-shaped tanks. From 7 DAH to 62 DAH this ratio decreased at a similar rate for both treatments ($F_{1;215}= 6.39$; $p \geq 0.05$)(Figure 5.5). The coefficient of variation for the ratio of mouth-gape to head depth at 16 DAH was 18% and 29 % for flat-bottomed and v-shaped tanks, respectively. At 62 DAH coefficient of variation decreased to 12 % and 6 % for flat-bottomed and v-shaped tanks, respectively. Least square regression models are listed in Table 5.2.

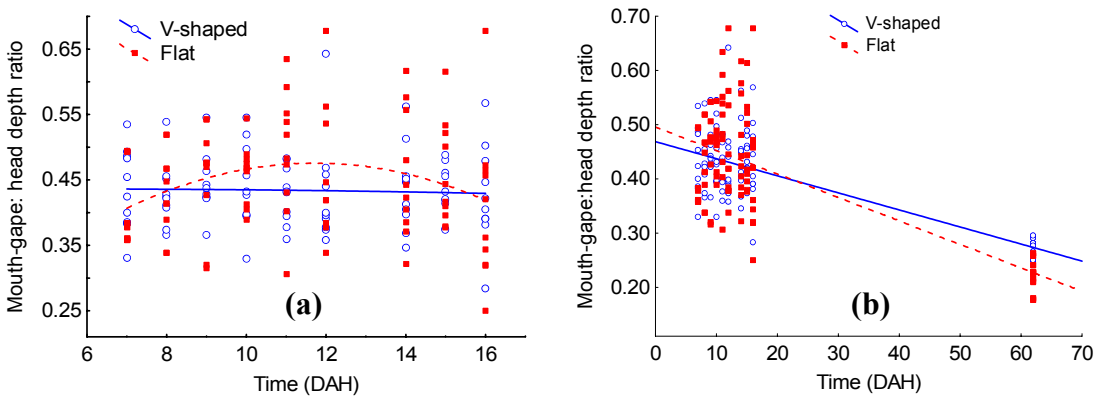


Figure 5.5. Mouth-gape to head depth ratio over time for goldfish larvae raised under two different conditions, a v-shaped tank bottom design which kept decapsulated *Artemia* cysts suspended (V-shaped) and a flat bottomed tank design in which cysts settled on the bottom of the tank (Flat). (a) represents ratio development until 16 DAH (n = 175) and (b) represents development until 62 DAH (n = 215).

Tail length to total length ratio

Tail length was shortest in relation to total length at 11 DAH in the flat-bottomed treatment after which it increased until 62 DAH. However, the tail length to total length ratio increased at a constant rate from 2 DAH to 62 DAH in fish from v-shaped tanks (Figure 5.6). Tank design significantly influenced the development of the ratio of tail length to total length from 2 DAH to 62 DAH ($F_{1;315} = 3.76$; $p \leq 0.05$). The ratio increased at a greater rate in fish from flat-bottomed tanks than those from v-shaped tanks. The coefficient of variation for flat- and v-bottomed tanks at 62 DAH was 12 % and 3 %, respectively, resulting in similar ratios for each treatment at 62 DAH ($t = 1.83$; $0.07 \leq p \leq 1.0$).

Total length to body depth ratio

The ratio of total length to body depth decreased linearly from 2 DAH to 62 DAH and there was no difference between treatments ($F_{1;315} = 0.39$; $p \geq 0.05$; Figure 5.7). Coefficient of variation of the total length to body depth ratio increased over time for fish reared in flat-bottomed tanks. The specimens with the highest and lowest total length to body depth ratio were from flat-bottomed tanks at 8, 9, 11, 12, 13, 15 and 16 DAH indicating higher variation in this treatment. At 16 DAH the average total length to body depth ratio was 4.93 ± 0.93 (CV = 19 %) and 5.00 ± 1.77 (CV = 35 %) for the v-shaped and flat-bottomed treatment, respectively. Although at 16 DAH juvenile goldfish reared in tanks with v-shaped bottoms were significantly larger than those from flat-bottomed tanks ($t = 13.3$; $p \leq 0.0001$), the ratio of body depth to total length did not differ significantly ($F_{1;315} = 0.39$; $p \geq 0.05$) between these treatments. The least square models describing the development of total length to body depth are listed in Table 5.2.

Survival of larvae at 62 DAH

Survival was high in both treatments. Fish from V-shaped tanks had a higher percent survival (88 %) than those from flat-bottomed tanks (74 %).

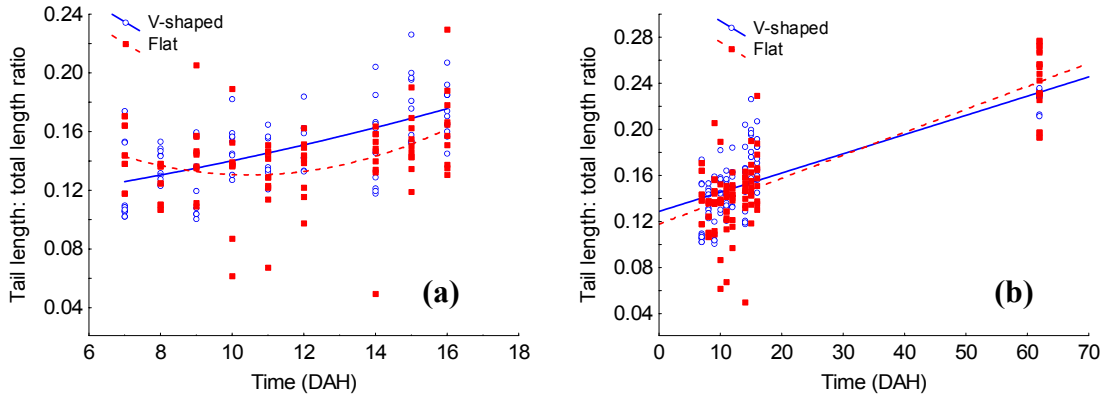


Figure 5.6. Tail length to total length ratio over time for goldfish larvae raised under two different conditions, a v-shaped tank bottom design which kept decapsulated *Artemia* cysts suspended (V-shaped) and a flat bottomed tank design in which cysts settled on the bottom of the tank (Flat). (a) represents ratio development until 16 DAH (n = 175) and (b) represents development until 62 DAH (n = 215).

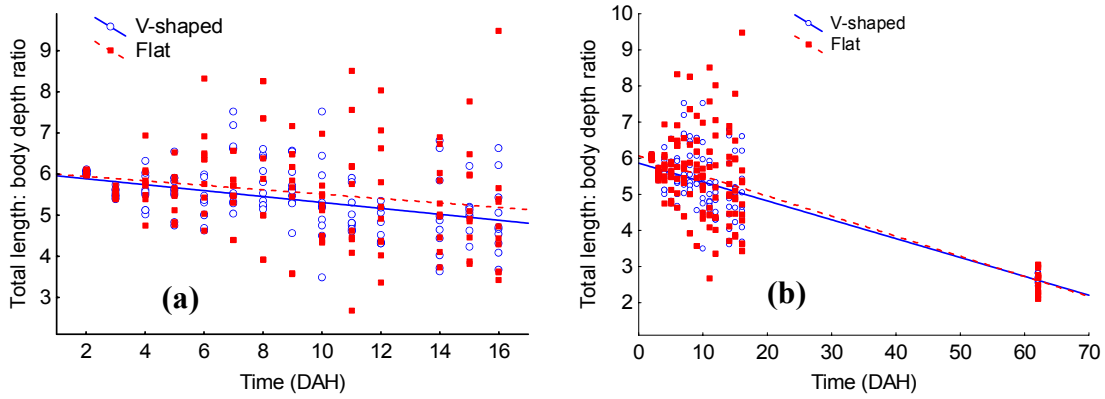


Figure 5.7. Total length to body depth ratio over time for goldfish larvae raised under two different conditions, a v-shaped tank bottom design which kept decapsulated *Artemia* cysts suspended (V-shaped) and a flat bottomed tank design in which cysts settled on the bottom of the tank (Flat). (a) represents ratio development until 16 DAH (n = 275) and (b) represents development until 62 DAH (n = 315).

Table 5.2: Least square regression models for the ratio of total length to standard length (**TL: SL**), mouth-gape to total length (**MG: TL**), mouth-gape to head depth (**MG: HD**), tail length to total length (**Tail: TL**) and, total length to body depth (**TL: BD**) for fish reared in either V-shaped (**V**) or flat-bottomed tanks (**Flat**). n is the number of goldfish in each sample and x is time in days.

Ratio	Treatment	Growth model	n
TL: SL (7 - 16 DAH)	V	$y = 1.1 + 0.002x + 0.0003x^2$	175
	Flat	$y = 1.3 – 0.02x + 0.001x^2$	175
TL: SL (7 - 62 DAH)	V	$y = 1.15 + 0.003x$	215
	Flat	$y = 1.13 + 0.003x$	215
MG: TL (7 – 16 DAH)	V	$y = 0.04 + 0.005x - 0.0002x^2$	175
	Flat	$y = 0.0001 + 0.01x - 0.0005x^2$	175
MG: TL (7 - 62 DAH)	V	$y = 0.07 + 0.00005x$	215
	Flat	$y = 0.07 + 0.00004x$	215
MG: HD (7 – 16 DAH)	V	$y = 0.4 + 0.0007x – 0.00006x^2$	175
	Flat	$y = 0.05 + 0.07x - 0.003x^2$	175
MG: HD (7 - 62 DAH)	V	$y = 0.47 - 0.003x$	215
	Flat	$y = 0.50 - 0.004x$	215
TAIL: TL (7 – 16 DAH)	V	$y = 0.1 + 0.003x + 0.0001x^2$	175
	Flat	$y = 0.2 - 0.02x + 0.001x^2$	175
TAIL: TL (7 - 62 DAH)	V	$y = 0.13 + 0.002x$	215
	Flat	$y = 0.11 + 0.002x$	215
TL: BD (7 – 16 DAH)	V	$y = 6.03 - 0.07x$	275
	Flat	$y = 6.05 - 0.05x$	275
TL: BD (7 - 62 DAH)	V	$y = 5.86 -0.05x$	315
	Flat	$y = 6.06 - 0.06x$	315

Discussion

Environmental effects on morphological plasticity and growth

Phenotypic plasticity has been defined as a change in phenotype as a response to differences in the rearing environment (Reznick & Yang, 1993) and foraging strategy (Meyer, 1987, 1990; Wimberger, 1992). This study has shown that in larval goldfish, phenotypic plasticity could be a response to differences in food availability. Goldfish larvae that were forced to feed on moving cysts in v-shaped tanks developed a larger mouth-gape in proportion to head depth than fish that fed on stationary cysts. This increased mouth-gape to head depth ratio could therefore have been an adaptation to facilitate the capture of moving cysts. Data from Chapter 4 also indicated that fish feeding on mobile prey (*Artemia* nauplii) had significantly larger mouth-gapes than those, which browsed cysts from the tank floor. Hegrenes (2001) reported similar plasticity in mouth-gape size and theorised that this could have been an adaptive response to the biomechanical functions necessary for prey capture. Since mouth-gape size has been linked to cannibalism (Cunha & Planas, 1999), it may be possible to reduce cannibalism in goldfish larvae through manipulation of diet and environmental conditions. While it is possible to induce morphometric change by manipulating diet type, it is difficult to determine whether these changes are adaptive (Reznick & Yang, 1993). For example, Reznick (1990) found that low food availability caused guppies *Poecilia reticulata* to reach sexual maturity at a smaller size and later age. This reduced size at maturity might have enhanced the individual's fitness in the changed environment which, in turn indicates that plasticity represents an adaptation which enables the fish to best exploit the environment in which it is living (Reznick & Yang, 1993).

The ratio of total length to body depth was not affected by tank design 62 days after hatching. This does not support findings by Hegrenes (2001) who suggested that benthic feeding sunfish *Lepomis humilis* have a deeper and less fusiform body than their conspecifics foraging in open water.

Feed ingestion

Water movement resulting in the movement of otherwise immobile prey items, increased the ingestion rate in ling *Molva molva* L. larvae (Løkkeborg *et al*., 2000), fathead minnow larvae *Pimephales promelas* (Landry *et al.*, 1995), cod larvae, *Gadus morhua*, herring larvae, *Clupea harengus* (Mackenzie & Kiorboe, 1995), juvenile gilthead sea bream *S. aurata* (Goldan *et* al., 1996), juvenile medaka *Oryzias laticeps* (Magnuson, 1962), larval walleye pollock *Theragra chalcogramma* (Megrey & Hinckley, 2001), and larval radiated shanny *Ulvaria subbifurcata* (Dower *et al.*, 1998). Similar results were found in this study. Cyst movement caused by up-welling current in v-shaped tanks may have increased food availability. It is therefore hypothesised that fish in v-shaped tanks had greater access to cysts than fish reared in flat-bottomed tanks. Lewis and Pedley (2001) reported for oceans and rivers that water movement increased the contact frequency and therefore ingestion of plankton in fish larvae. However, while the time taken to capture prey will be reduced by water current, capture success depends on the swimming ability of the fish as well as its visual acuity (Lewis & Pedley, 2001). Capture success, expressed as the percentage of successful feeding strikes, is low at first feeding in most fish larvae but rises rapidly as fish grow (Houde & Schekter, 1980). This is due to an increase in feeding experience (Colgan *et al*., 1986) and improved manoeuvrability

with increased length (Hurst, 1994). In Chapter 7, prey preference for either small, drifting *Artemia* cysts or larger, more mobile *Artemia* nauplii will be assessed.

Size variation in goldfish juveniles

Increased size variation in fish from flat-bottomed tanks may have resulted in a greater number of aggressive interactions between larvae. Although this experiment did not study agonistic behaviour, size variation has been reported as a catalyst for agonistic behaviour in other fish species (Ruzzante, 1994) such as African catfish *C. gariepinus* (Hecht & Appelbaum, 1988) and koi carp, *C. carpio* (Van Damme *et al*., 1989). In common carp dominant individuals aggressively denied subordinate fish access to food (Nakamura & Kasahara, 1955). A possible explanation for the large size variation among fish reared in flat-bottomed tanks is that some fish are better foragers than others. Individual differences in food acquisition have been reported as a cause of high size variation (Umino *et al*., 1997; Irwin *et al*., 2000). Some slow-growing goldfish may not have eaten enough cysts from the bottom of flat-bottomed tanks. Unsuccessful browsing has been reported for carp larvae, *C. carpio* by Vanhaecke *et al*. (1990) who showed that they were not effective browsers and did not pick many cysts from the bottom of the culture tank.

Commercially produced fish are commonly size-graded to overcome the disadvantages of size variation (Goldan *et al*., 1998). An alternative approach to size-grading is to understand the mechanisms that cause size variation and then to manipulate rearing conditions in a way which would reduce this size variation. For example, this study has shown that by changing from static to mobile prey caused a reduction in size variation in goldfish juveniles.

Practical aspects of flat-bottomed and v-shaped tanks

In addition to allowing for suspension of cysts, the v-shaped tank with a perforated inflow pipe along the tank bottom created conditions that did not provide a substrate for fungal contamination of uneaten food or faeces. Flat-bottomed tanks became contaminated by fungal growth. This became more problematic once dry food was added from 17 DAH, as the outflow screens on each flat-bottomed tank became clogged regularly with a mixture of fungus and uneaten food. Daily siphoning of v-shaped tanks was made easy when the water flow was turned off and waste products collected in the middle of the tank. Thus, in commercial culture systems, time spent maintaining culture tanks and size-sorting fish could be reduced through the use of v-shaped tanks. In addition, keeping cysts suspended in the water column significantly increased the growth of larval goldfish. Table 5.3 compares the different tank designs.

Table 5.3. Comparison of v-shaped and flat-bottomed treatments at 62 DAH.

Variable	V-shaped tank	Flat-bottomed tank
Fish size at 62 DAH	larger	smaller
TL: BD ratio at 62 DAH	same	same
Survival at 62 DAH	88 %	74 %
Coefficient of variation for fish size	5 % (total length)	12 % total length
Tank cleaning	easy	time consuming
Fungal contamination	no growth	plenty of growth

Chapter 6

The effect of feeding rate of decapsulated *Artemia* cysts on larval and juvenile goldfish growth and survival

Introduction

Results from Chapter 4 suggested that feeding decapsulated *Artemia* cysts to goldfish larvaeresulted in better growth than feeding either *Artemia* nauplii or a mixed live /dry diet of nauplii and dry food. Thus, the effect of feeding different quantities of decapsulated *Artemia* cysts on growth, size distribution and survival was tested in this study. Decapsulated cysts have been suggested as an alternative to *Artemia* in the rearing of larval carp, *Cyprinus carpio* (Vanhaecke *et al.*, 1990) as well as other cyprinids including tench *Tinca tinca*, grass carp *Ctenopharyngodon idella*, and ide *Leuciscus idus* (Jähnichen & Kohlmann, 1999). Dhert *et al*. (1997) found that growth and survival of guppies *Poecilia reticulata* was significantly better in fish fed decapsulated cysts than in those fed *Moina*. Good survival rates for fish fed decapsulated cysts have also been demonstrated for cyprinids such as *C. idella* (Vanhaecke *et al.*, 1990) and *C. carpio* (Jähnichen & Kohlmann, 1999) and survival rates as high as 95 % have been recorded for sturgeon larvae, *A. oxyrinchus desotoi* (Bardi *et al*., 1988).

Artemia cysts are a good alternative to live *Artemia* as a diet for larval fish. Under intensive rearing conditions there is a need to quantify the amount of food required for best growth in order to prevent a possible increase in size variation at low feeding rates as well as to reduce the deterioration of water quality resulting from uneaten food (Kestemont, 1995). Low

feeding rates are known to cause variation in growth and extended duration of the larval stage in herring *Clupea harengus* (Werner & Blaxter, 1980), and in carp larvae, *C. carpio,* under-feeding resulted in slow growth and increased cannibalism (Bryant & Matty, 1981). Size variation induces agonistic behaviour and is often caused by the lack of an optimal feeding level (Abi-Ayad & Kestemont, 1994; Goldan *et al*., 1998). This may lead to growth suppression of a proportion of the population.

Improper harvesting and processing of *Artemia* cysts does not affect their nutritional value. Thus, poor-hatching and less expensive cyst products can be utilised for the culture of carp larvae (Vanhaecke *et al*., 1990). Determining that feeding level that results in the best growth and survival in goldfish larvae will reduce production costs in intensive aquaculture systems, optimise larval growth and potentially reduce size variation in goldfish.

Aims and objectives

The aim of this trial was to test five different feeding levels of decapsulated *Artemia* cysts and their effect on growth and size variation from first feeding to 16 days after hatching (DAH). Objectives:

- To determine the effect of cyst feeding level on the growth rate of goldfish larvae during the first 16 DAH;
- to determine to what extent feeding rate affects the variation of total length, mouth-gape and body depth at 16 DAH;
- to determine if feeding rate has a significant effect on the ratio of mouth-gape to total length and body depth to total length at 16 DAH; and
- to determine percentage survival of fish for each treatment at 16 DAH.

Materials and methods

Six hours after hatching, larval goldfish were transferred into the 800-L recirculating aquarium system that comprised 36 glass tanks, a trickle filter, and a submerged biological filter as described in Chapter 2. Temperature was maintained at 23 ± 1.5˚C by a central heater-thermostat unit in the system sump. Photoperiod was maintained at 12L: 12D by L18 W/72 Biolux tubes (Osram). Aeration was supplied to the pump sump and oxygenated water delivered to each V-shaped tank via a perforated pipe along the tank bottom at 0.6 L /min. Twenty V-shaped tanks (Chapter 2; Figure 2.2) were used for study.

Each treatment had four replicate tanks randomly assigned to the system and fish were stocked into each tank at twelve fish per litre. Two days after hatching, fish were fed 31, 62, 93, 124, or 155 decapsulated *Artemia* cysts per fish per day. The required number of prey items was calculated using the method described in Chapter 2, and then pipetted into each tank. Fish were fed five times per day (08:00h, 10:00h, 12:00h, 14:00h and 16:00h) until 8 DAH and then three times per day (08:00h, 12:00h and 16:00h) until the end of the experiment. Each day before first feeding, water inflow was turned off allowing suspended solids and any uneaten cysts to settle before being siphoned from the tank bottom. Dead fish were removed and counted daily.

A random sample of five fish was removed for measurement from each tank each morning before first feeding and replicates from the two tanks of each treatment were pooled. Two tanks for each treatment were not sampled in order to determine percentage survival at the end of the study. Total length and body depth were measured in millimetres to two decimal places, prior to the measurement of mouth-gape for each specimen (see Chapter 2 for a detailed description of the materials and methods).

Statistical analysis

Analysis of covariance (ANCOVA) was used to test for difference in changes of continuous data over time such as total length and body depth: total length ratio for each treatment. The non-parametric Kruskall-Wallis ANOVA by ranks test was used to compare goldfish morphometric measurements, the ratio of total length to mouth-gape and the ratio of total length to body depth of fish fed different amounts of cysts. This was followed by a pair-wise comparison of the data using the Mann-Whitney U test and a Bonferroni-corrected conservative p-value.

The coefficient of variation (CV) is a measure of relative dispersion and was used to compare variation within the population at different times. CV is described by:

$Cv = \frac{\sigma}{\bar{\chi}} \bullet 100$; where σ is the standard deviation, and $\bar{\chi}$ is the mean.

Results

Growth in total length was significantly affected by the number of cysts fed ($F_{4,690}$ = 62,4; p < 0.0001) (Figure 6.1). Growth increased (p < 0.001) as the number of cysts fed to each fish per day increased and fish fed 166 cysts per fish per day grew significantly better than fish from all other treatments. Fish fed 93 and 124 cysts per fish per day did not differ significantly from each other ($F_{1,276}$ = 3.88; p ≥ 0.05). All other treatments differed significantly from each other. Growth was linear for all treatments for the first 16 DAH. Correlation coefficients (r^2) for the growth models increased with the number of cysts fed. At 16 DAH the coefficient of variation decreased with an increase in feeding level and was 10 %, 9 %, 8 %, 8%, and 7 % for fish fed 31, 62, 93, 124, and 155 cysts per fish per day.

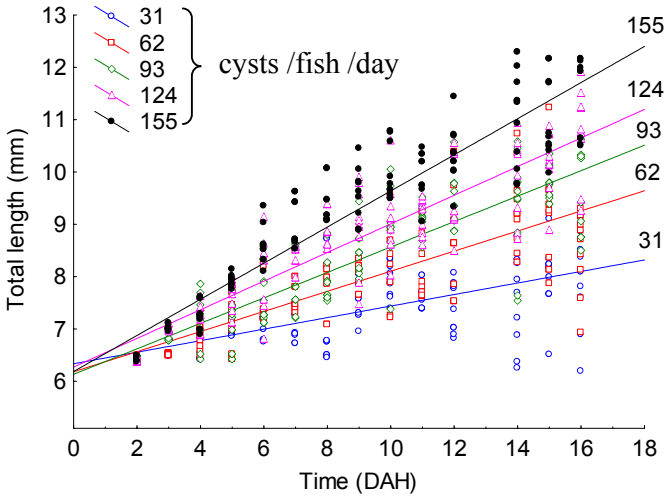


Figure 6.1 Increase in total length over time from 2-16 days after hatch (DAH) at five feeding levels, 31, 62, 93, 124, and 155 cysts /fish /day. n = 140 for each treatment.

Table 6.1 Growth models for goldfish larvae fed either 31, 62, 93, 124, and 155 cysts /fish /day from 0-16 days after hatching (DAH). Different superscript denote a significant difference between treatments at the Bonferroni-corrected level of $p \leq 0.005$. n = 140 for each treatment. y = total length (mm); x = day after hatching; r^2 = correlation coefficient.

Feeding rate (cysts /fish /day)	Growth model	r^2
31	$y = 6.33 + 0.11x$ [a]	43 %
62	$y = 6.18 + 0.19x$ [b]	65 %
93	$y = 6.13 + 0.24x$ [c]	78 %
124	$y = 6.27 + 0.27x$ [c]	82 %
155	$y = 6.18 + 0.35x$ [d]	87 %

The ratio of mouth-gape to total length did not differ significantly between feeding levels at 16 DAH (Kruskal-Wallis test: $H_{4,49} = 6.88$; $0.14 \geq p \geq 0.05$) (Figure 6.2). The coefficient of variation was lowest at 2 % in fish fed 155 cysts per fish per day.

The ratio of body depth to total length differed significantly between feeding levels (Kruskal-Wallis test: $H_{4;49} = 29.0$; $p < 00001$) (Figure 6.3; Figure 6.4). The body depth to total length ratio remained constant over time in fish fed 31 cysts per day but increased with time and an increase in feeding level for the other treatments (Figure 6.3). Fish fed 31 cysts per day had significantly smaller body depth in relation to total length than fish fed 93, 124 or 155 cysts per day ($p \leq 0.001$) but did not differ significantly from those fed 62 cysts per day (Mann-Whitney U test: $Z_{1,19} = 0.68$; $p \geq 0.05$). Coefficient of variation of the body depth to total length ratios decreased from 14 % in fish fed 62 cysts per day to 3 % and 4 % for fish fed 124 and 155 cysts per day, respectively.

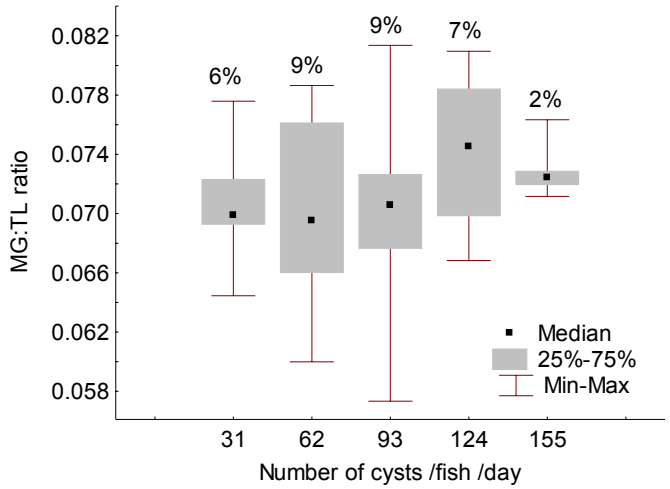


Figure 6.2 Ratio of mouth-gape to total length at 16 days after hatching for fish fed 31, 62, 93, 124, and 155 cysts /fish /day. Coefficient of variation is shown for each plot as the percentage value. n = 10 for each treatment.

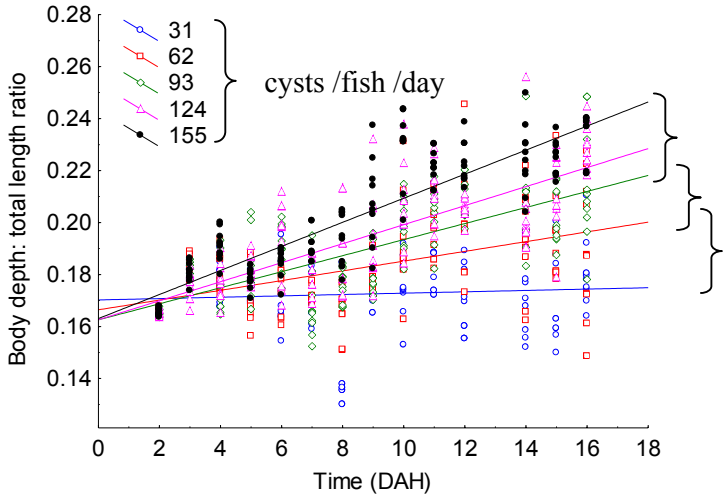


Figure 6.3 Development of the body depth: total length ratio for fish fed either 31, 62, 93, 124, or 155 cysts per fish per day for the first 16 days after hatching (DAH). Similar growth curves are grouped by brackets at $p \leq 0.001$. n = 140 for each treatment.

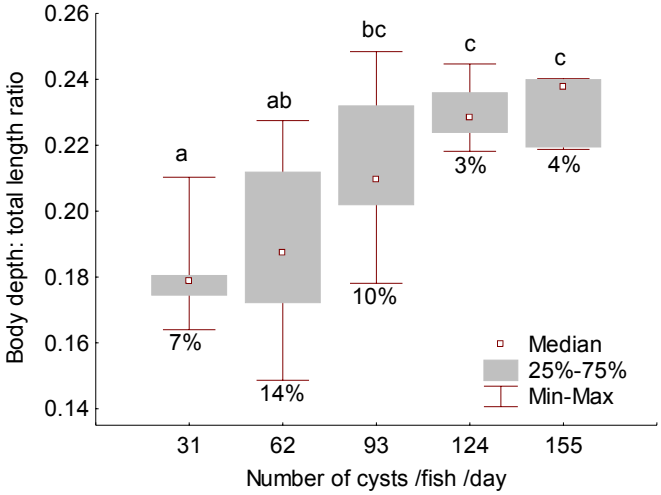


Figure 6.4 Ratio of body depth to total length at 16 days after hatching (DAH) for fish fed either 31, 62, 93, 124, or 155 cysts per fish per day. Different letters indicate significant difference at $p < 0.001$ and percentage values represent the coefficient of variation for each treatment. n = 10 for each treatment.

Percentage survival increased with an increase in feeding level (Table 6.2) with 89 % of fish fed 155 cysts per fish per day surviving at 16 DAH.

Table 6.2 Percentage survival of fish fed 31, 62, 93, 124, or 155 decapsulated *Artemia* cysts per fish per day until 16 days after hatching.

Feeding level (cysts per fish per day)	Percentage survival
31	49 %
62	61 %
93	80 %
124	84 %
155	89 %

Discussion

Fish grew better as feeding level increased but results indicated that even faster growth might be achieved with a higher feeding rate than the maximum of 155 cysts per fish per day tested for this study. This was because fish fed 155 cysts daily grew significantly better than those fed at lower feeding levels (Figure 6.1). Vanhaecke *et al*. (1990) found that the number of cysts fed to carp could be reduced by 25 % and 35 % after one and two weeks culturing, respectively. However, the authors did not provide growth curves for their study and since Vanhaecke *et al*. (1990) also reported that carp larvae did not browse many cysts from the tank bottom, their results could not be used for comparison. Furthermore, the up-welling water movement in this study prevented cysts from settling on the tank bottom.

Size variation

Low feed supply increased size variation in goldfish larvae and juveniles. This relationship has been reported for goldfish (Abi-Ayad & Kestemont, 1994) and the gilthead sea bream *Sparus aurata* (Goldan *et al*., 1998). Hecht & Pienaar (1993) showed that a wide size variation, caused primarily by reduced food availability and low feeding frequency, resulted in increased agonistic behaviour among larvae of African catfish *C. gariepinus*. Size variation was 15 % lower in fish fed 93 or more cysts per fish per day compared to fish fed less than 93 cysts per day. Therefore, it may be possible to manage size variation in goldfish larvae and juveniles by maintaining a high feeding level. Furthermore, better growth was achieved at feeding rates exceeding 93 cysts per fish per day. In gilthead sea-bream *Sparus aurata* culture a wide size variation was found to be disadvantageous as growth suppression of small individuals occurred (Ruzzante, 1994). While size grading is beneficial in the long term, it is stressful to the fish, labour intensive and needs to be done

regularly. Therefore, control of size variation in fish larvae and juveniles by feeding at a minimum level has applications for larval rearing.

Delayed metamorphosis

Feeding goldfish larvae less than 93 cysts per fish per day delayed the completion of metamorphosis by one to two days. Delayed development and extended larval stages at low feeding levels has been reported for larval striped bass *Morone saxatilis* (Eldridge *et al*., 1981), larval herring *Clupea harengus* (Werner & Blaxter, 1980), and Atlantic cod larvae *Gadus morhua* (Puvanendran & Brown, 1999). The problem associated with under-feeding carp larvae was demonstrated by Bryant & Matty (1981) who showed that impaired growth was associated with an increase in cannibalism. The rate of cannibalism was low for all treatments of this study as most dead fish were recovered whole or had been scavenged after death.

Larval survival at different feeding levels

Based on the data from this experiment it is recommended that goldfish larvae be fed at least 93 cysts per fish per day to achieve high survival (≥ 80%) and good growth. Other studies have calculated ration size as a percentage of body weight. For example, survival of African catfish *C. gariepinus* survival increased from 53 % to 93 % when the feeding ration was increased from 20 % to 85 % of body weight (Hecht & Pienaar, 1993). Increased survival at high feeding levels has been demonstrated for common carp *C. carpio* (Bryant & Matty, 1980) and larval striped bass *M. saxatilis* (Eldridge *et al*., 1981).

Morphological plasticity

Previous experiments determined that diet type modified the development of goldfish (Chapter 4). Data from this study showed that development of mouth-gape was not affected by feeding rate in goldfish larvae and juveniles. Thus, mouth-gape development appears to be modified by food type but not by food availability. This indicates that plasticity is driven by the feeding strategy necessary to capture different prey types. Diet-induced polymorphisms have been described for many fish species such as sticklebacks *Gasterosteus aculeatus* (Schluter, 1993, 1995), barbus species (Nagelkerke, 1997), and the Arctic charr *Salvelinus alpinus* (Magnusson & Ferguson, 1987), but no mention is made of feeding level having a differential effect on the development of larvae.

Body depth in relation to total length increased with an increase in feeding level and is used as a measure of condition. The ratio of body depth to total length has been used as an accurate method for describing the condition of larval Atlantic cod *G. morhua* (Koslow *et al*., 1985; Yin & Blaxter, 1987). Deeper-bodied fish are considered to be in better physical condition than thinner fish. Fish fed 31 or 62 cysts per day were thin compared to fish fed 93 or more cysts per day. This supports the hypothesis that feeding less than 93 cysts per fish per day limited the growth and development of juvenile goldfish. Fish fed 155 cysts per fish per day grew fastest (Figure 6.1); had the highest survival rate (Table 6.2); were in the best condition (Figure 6.4); and had the lowest size-variation. It was therefore concluded that, within the range of feeding levels of cysts tested, 155 cysts per fish per day gave the best results at 23 ± 1.5°C and a population density of 12 fish per litre.

Chapter 7

Larval and juvenile preference for either decapsulated *Artemia* cysts or *Artemia* nauplii: ingestion and rejection patterns according to diet type

Introduction

Experiments from this thesis (Chapter 4) indicated that fish fed decapsulated *Artemia* cysts grew faster than those fed either *Artemia* nauplii or a mixed live/dry diet. This study was designed to observe the feeding behaviour of goldfish larvae and juveniles to determine if they had a preference for either *Artemia* nauplii or *Artemia* cysts. This may help to interpret the results of previous chapters.

Analysis of stomach content has been used to quantify feed intake (Jobling *et al*., 2001). However, gut content analysis cannot tell when and how quickly prey items were ingested (Jobling *et al*., 2001). For these reasons, behaviour observations have been conducted for decades to study feeding behaviour of fish under experimental conditions (Jobling *et al*., 2001). During behaviour observations a single fish can be observed over a defined period. An understanding of feeding behaviour under culture conditions may help provide a rearing environment that allows for high ingestion rates and best availability of preferred food items.

Taste and feeding behaviour in fish

Feeding in fish is dependent on their sensory capacity to locate food, their ability to capture, handle and ingest food items, and their physiological and biochemical ability to digest and metabolise the ingested nutrients (Kestemont & Baras, 2001). The interaction between these factors combined with environmental conditions and social behaviour influences feeding success in fish.

Feeding behaviour in fish has been divided into three phases beginning with an alerting or arousal phase, followed by an appetitive phase where potential food items are identified and located, and concluded by feed intake leading to ingestion or rejection of the prey item (Jones (1992) cited in Lamb, 2001). The arousal phase serves as a primer for the appetitive stage and is often initiated by chemical detection of food items or amino acids in the water (Lamb, 2001). For example L-alanine, L-arginine and L-proline have been shown to stimulate feeding behaviour in goldfish (Lamb & Finger, 1995). Continued stimulation during the arousal phase resulted in searching behaviour that signalled the appetitive phase of feeding which included swimming patterns directed towards locating the chemical stimulus. Bardi *et al*. (1998) found that the addition of live brine shrimp, and especially their decapsulated cysts, elicited a strong swimming, search, and feeding stimulus in sturgeon larvae, *Acipenser oxyrinchus desotoi*. Survival rates were in excess of 95 % in larvae that selected cysts and were lower for the other diets tested.

Once a potential food source has been located, it is taken into the mouth where palatability is assessed. Goldfish have a highly developed vagal gustatory system and may have developed complex prey item selection mechanisms (Figure 7.1; Lamb, 2001). Feeding behaviour in goldfish consists of taking food into the mouth, followed by sorting and

identifying palatability of the food item and culminating in either ingestion of the food item or rejection if it was found to be unpalatable (Lamb, 2001). Results from anatomical, physiological and behavioural studies should be combined to provide a systematic analysis of the feeding behaviour and dietary requirements of a species (Lamb, 2001).

Abundance and the temporal and spatial distribution of food influence feed intake in fish coupled with biotic factors such as social interactions and competition for food. Competition for food increases when food is restricted (Kestemont & Baras, 2001). In this study food was supplied in apparent excess of satiation to reduce the effect of competition on food preference and to limit potential agonistic behaviour between siblings.

Capture success is defined as the percentage of successful feeding strikes and is low at first

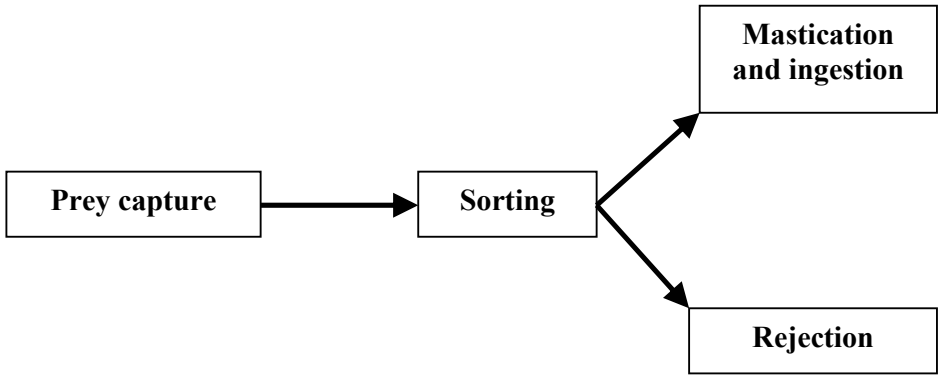


Figure 7.1 Feeding behaviour pattern of goldfish. Food is sucked into the oral cavity and is held in the pharynx where it is sorted. Food is then moved to the oesophagus for ingestion, while non-food-items or those deemed unpalatable, are rejected through the mouth and opercular slits (Adapted from Lamb & Finger, 1995).

feeding in most fish larvae but rises during early development (Houde & Schekter, 1980) due to increased feeding experience (Colgan *et al*., 1986) and improved manoeuvrability (Hurst, 1994). In Atlantic cod larvae, *Gadus morhua*, reared at different prey densities, there was no significant difference in agonistic behaviour during the first week after

hatching. However, after two weeks those larvae reared at the lower prey concentration swam for longer periods and attacked more prey items with a lower success rate (Puvanendran & Brown, 1999). This suggested that the cod larvae attacked any prey encountered but rejected food more often (Opuszynski & Shireman, 1993). Herring larvae were also found to attack more prey items with a high rate of rejection at low prey densities (Munk & Kiørboe, 1986). In snapper larvae, *Pagrus auratus*, aggressive contacts were more frequent during feeding than during resting and swimming (Hecht *et al*., 1996).

Prey selection in larval fish

Visual acuity, visual threshold and spectral sensitivity, prey contrast and its shape and mobility affect prey selection in fish larvae (Holm, 1986; Khadkar & Ramakrishna Rao, 1986; Cunha & Planas, 1999). In addition, prey selection in larvae is affected by prey density (Emlen, 1966; Chakrabarti & Jana, 1991; Schael *et al*., 1991; Opusszynski & Shireman, 1993), prey distribution (Jakobsen & Johnsen, 1987), light intensity (Mills *et al*., 1986; Mookerji & Rao, 1993), experience (Ringler, 1979), hunger level (Kislalioglu, 1976), and intra-specific competition (Rubinstein, 1981).

The aim of this study was to describe prey preference and agonistic behaviour of goldfish larvae and juveniles fed a mixture of decapsulated *Artemia* cysts and instar I *Artemia* nauplii. Feeding behaviour was recorded at 3, 10 and 17 days after hatching (DAH), and the following objectives were set:

- To quantify the number of cysts and *Artemia* ingested per minute for the first ten minutes of feeding at 3, 10 and 17 DAH;

- to determine if there was a prey item preference throughout the larval and juvenile period;
- to determine the rejection rate for any of the two prey items on 3, 10 and 17 DAH; and
- to determine to what extent agonistic behaviour affected feeding success and how the behaviour of the fish changed with age.

Materials and methods

Six hours after hatching, larval goldfish were transferred into an 800-L recirculating aquarium system that comprised 36 glass tanks, a trickle filter, and a submerged biological filter as described in Chapter 2. Temperature was maintained at 23 ± 1.5˚C by a central heater-thermostat unit in the system sump. Photoperiod was maintained at 12L:12D by L18 W/72 Biolux tubes (Osram). Aeration was supplied to the pump sump and oxygenated water delivered at 0.6 L /min to each V-shaped tank via a perforated pipe along the tank bottom.

Fish were stocked into two tanks at 25 fish L^{-1}. From two days after hatching, the fish were fed decapsulated *Artemia* cysts and instar I *Artemia* nauplii at a rate of 125 cysts /fish /day and 125 nauplii /fish /day. The required number of prey items was calculated using the method described in Chapter 2. Fish were fed five times per day at 08:00h, 10:00h, 12:00h, 14:00h, and 16:00h until 8 DAH and then three times per day at 08:00h, 12:00h, and 16:00h until the end of the experiment. Each day before first feeding, water inflow was turned off allowing solids and any uneaten cysts to settle before they were siphoned from the tank bottom.

A fluorescent tube (L18 W/72 Biolux tube) above each tank provided lighting. A dark sheet of rigid plastic with a small window was placed in front of the tanks. Fish were observed through the window to limit any possible disturbance caused by the observer. Definitions of the terms used for this study include:

- "capture" – occurs when prey is taken into the fish's mouth before it decides whether or not to ingest or reject the prey item;

- "ingestion" – describes when the prey item is swallowed;
- "rejection" – describes when then captured prey item is released back into the water and not swallowed; and
- "rejection rate" – is the number of prey items rejected out of the total number captured.

Observations included: (1) feed intake expressed as the number of feed items captured per minute; (2) frequency of ingestion or rejection of prey items; and (3) frequency of agonistic events. Agonistic encounters included nipping i.e., swimming into the side of another fish in an attempt to bite it, and chasing, defined as swimming after a fish that was trying to avoid the aggressor.

At each feeding, fish were fed 25 cysts and 25 nauplii per fish until 8 DAH and then 52 cysts and 52 nauplii per fish three times per day. At 08:00h on 3, 10 and 17 DAH fish were fed before their behaviour was recorded. In order to reduce the effect of possible patchiness of food items, each tank was divided into ten sections (Figure 7.2). One fish was chosen randomly from each numbered section of the tank and observed for one minute. Fish behaviour was recorded using computer software. Ten one-minute observations were recorded for each of the two replicates at 3, 10 and 17 DAH.

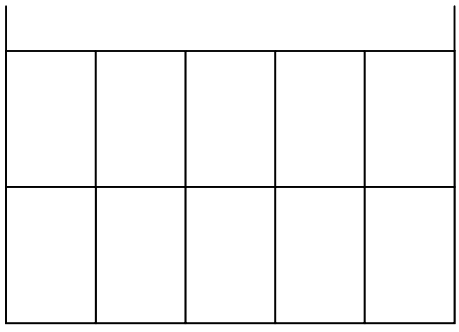


Figure 7.2 Observation tank viewed from the side showing the ten sections from which an individual was chosen at the start of each one-minute observation.

Statistical analysis

Least square regression breakpoint analysis was used to estimate if and when satiation was reached. Having identified this time, data were divided into the period before and after satiation. Frequency of ingestion, rejection, agonistic behaviour and prey selection was compared for the periods before and after satiation for 3, 10 and 17 DAH using Chi square analysis. Replicates were pooled together for Chi square analysis and if $p \geq 0.05$ an equal distribution was assumed and frequencies of events were considered to be similar for the two time periods. If behaviour was found to differ between 3, 10, and 17 DAH Chi square tests were used to determine where the differences occurred. For these repeated χ^2 analyses the p-value was adjusted using a Bonferroni – correction to $p \leq 0.017$ for each test.

Results

Breakpoint analysis indicated that fish reached satiation after five minutes of feeding at 3 DAH, 10 DAH and 17 DAH (95 %, 90 % and 95 % of the variance explained, respectively). Thus, data were separated into two sets; 1-5 minutes (before satiation) and 6-10 minutes. The total number of events for each five-minute period is presented in Table 7.1. At 3 DAH, 85 % of the food items ingested over the 10-minute period were eaten in the first five minutes and at 10 and 17 DAH this value was 86 % and 88 %, respectively.

Behaviour before satiation (1-5 min)

The number of prey items ingested increased significantly from 3 DAH to 17 DAH ($p \leq 0.012$). At 10 DAH, the number of prey items ingested did not differ significantly from 3 or 17 DAH ($p \leq 0.055$). The number of food items rejected peaked at 10 DAH and then decreased by 17 DAH ($p \leq 0.001$) when the rejection rate was 22 %. The frequency of agonistic encounters increased significantly ($p \leq 0.001$) from two encounters at 3 DAH to 39 at 17 DAH.

Behaviour after satiation (6-10 mins)

There was no significant difference in the number of prey items ingested between the three days of observation ($p \leq 0.867$). 3-4 prey items were ingested after satiation (per five minutes) at 3, 10 and 17 DAH. However, significantly more prey items were rejected at 3 DAH than 17 DAH ($p \leq 0.01$). At 10 DAH the number of prey items rejected was similar to 3 and 17 DAH ($p \geq 0.05$). The frequency of agonistic encounters increased significantly with time ($p \leq 0.001$), increasing from two to 43 encounters from 3 DAH to 17 DAH,

respectively. Percent rejection of food items for this period decreased from 88 % (3 DAH) to 75 % (17 DAH).

Table 7.1: Number of prey items ingested, number of agonistic events, number of rejected food items and percentage rejection prior to (1-5 mins), and after (6-10 mins) satiation at 3, 10, and 17 DAH. Chi square (χ^2) p-values were determined from the summed totals for each treatment.

		3 DAH	10 DAH	17 DAH	p-value
1-5 min	**Total ingested**	17	24	29	0.055
	Agonistic encounters	2	20	39	0.000
	Total rejected	15	32	8	0.000
	% prey items rejected	47 %	57 %	22 %	
6-10 min	**Total ingested**	3	4	4	0.867
	Agonistic encounters	2	30	43	0.000
	Total rejected	23	15	12	0.021
	% prey items rejected	88 %	79 %	75 %	

On 3, 10 and 17 DAH fish ingested a significantly greater number of prey items in the period before satiation ($p \leq 0.001$) than thereafter (Figure 7.5). The frequency of agonistic encounters remained similar throughout the observations on each day despite increasing from 3 DAH to 17 DAH (Figure 7.6).

Feed preference

The total number of food items consumed within ten minutes increased significantly ($p \leq 0.05$) from 20 to 28 and then to 33 for 3, 10, and 17 DAH, respectively. Decapsulated *Artemia* cysts were more frequently ingested and captured ($p \leq 0.01$) (Figure 7.4) than *Artemia* nauplii.

Before satiation (1-5 minutes of feeding)

At 3 and 10 DAH, fish rejected a higher percentage of decapsulated cysts ($p \leq 0.05$) than nauplii (Table 7.2, Figure 7.5). However, they ingested a greater number of decapsulated cysts than *Artemia* nauplii. At 17 DAH the rejection rate of *Artemia* cysts was similar to that of nauplii ($p \geq 0.05$). Significantly fewer cysts were captured at 17 DAH than at 10 DAH ($p \leq 0.05$) but due to the lower rejection rate of cysts at 17 DAH, more cysts were ingested.

6-10 minutes of feeding

At 3, 10 and 17 DAH, a similar number of decapsulated *Artemia* cysts and *Artemia* nauplii were ingested (Figure 7.4) ($p \geq 0.05$). Percent rejection of both cysts and nauplii in the period after satiation (Table 7.2) ranged from 67 % to 80 % rejection for cysts and 50 % to 83 % rejection for nauplii.

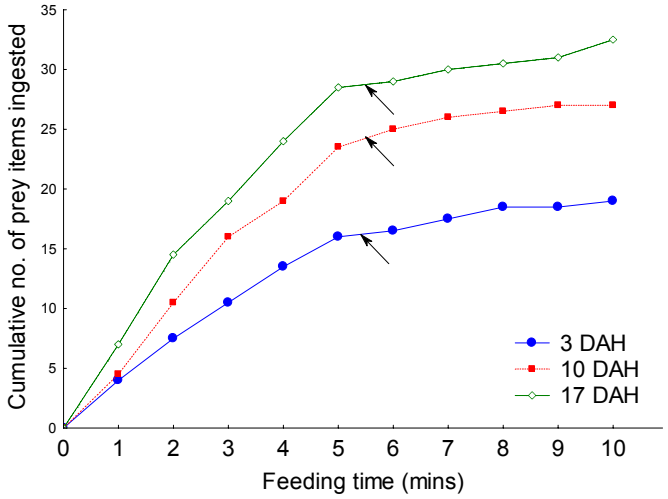


Figure 7.3 Cumulative number of prey items ingested at 3, 10 and 17 DAH during the first 10 minutes of feeding. The arrows indicate results of breakpoint analysis and are estimations of the time at which satiation was reached.

Table 7.2 Percentage rejection rates of cysts and nauplii by goldfish larvae and juveniles before (1-5min) and after (6-10 min) satiation at 3, 10 and 17 days after hatching (DAH). The figures in brackets represent the number of cysts or nauplii captured.

	1-5 min		**6-10 min**	
	cysts	**nauplii**	**cysts**	**nauplii**
3 DAH	63 % (27)	22 % (9)	75 % (20)	50 % (4)
10 DAH	60 % (35)	55 % (20)	67 % (15)	83 % (6)
17 DAH	24 % (21)	33 % (15)	80 % (10)	57 % (7)

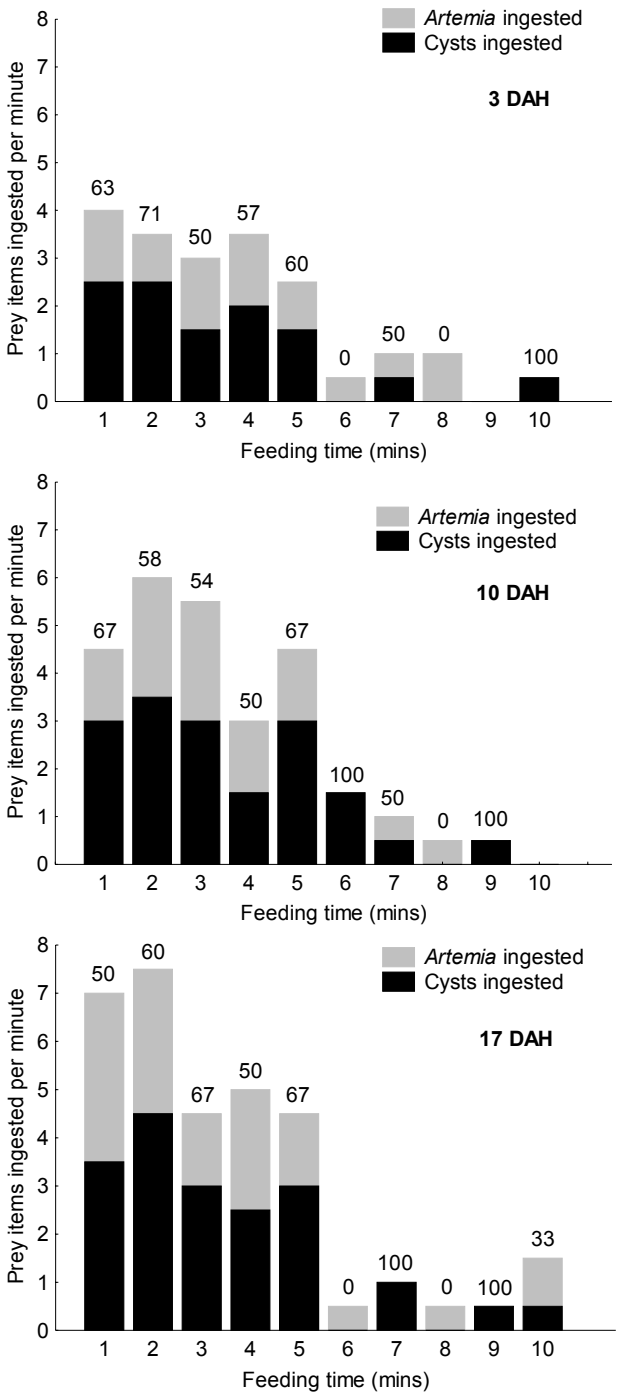


Figure 7.4 Comparison of the average number of decapsulated *Artemia* cysts and *Artemia* nauplii ingested during the first 10 minutes of feeding at 3, 10 and 17 DAH. The values on top of each column indicate the percentage of cysts ingested.

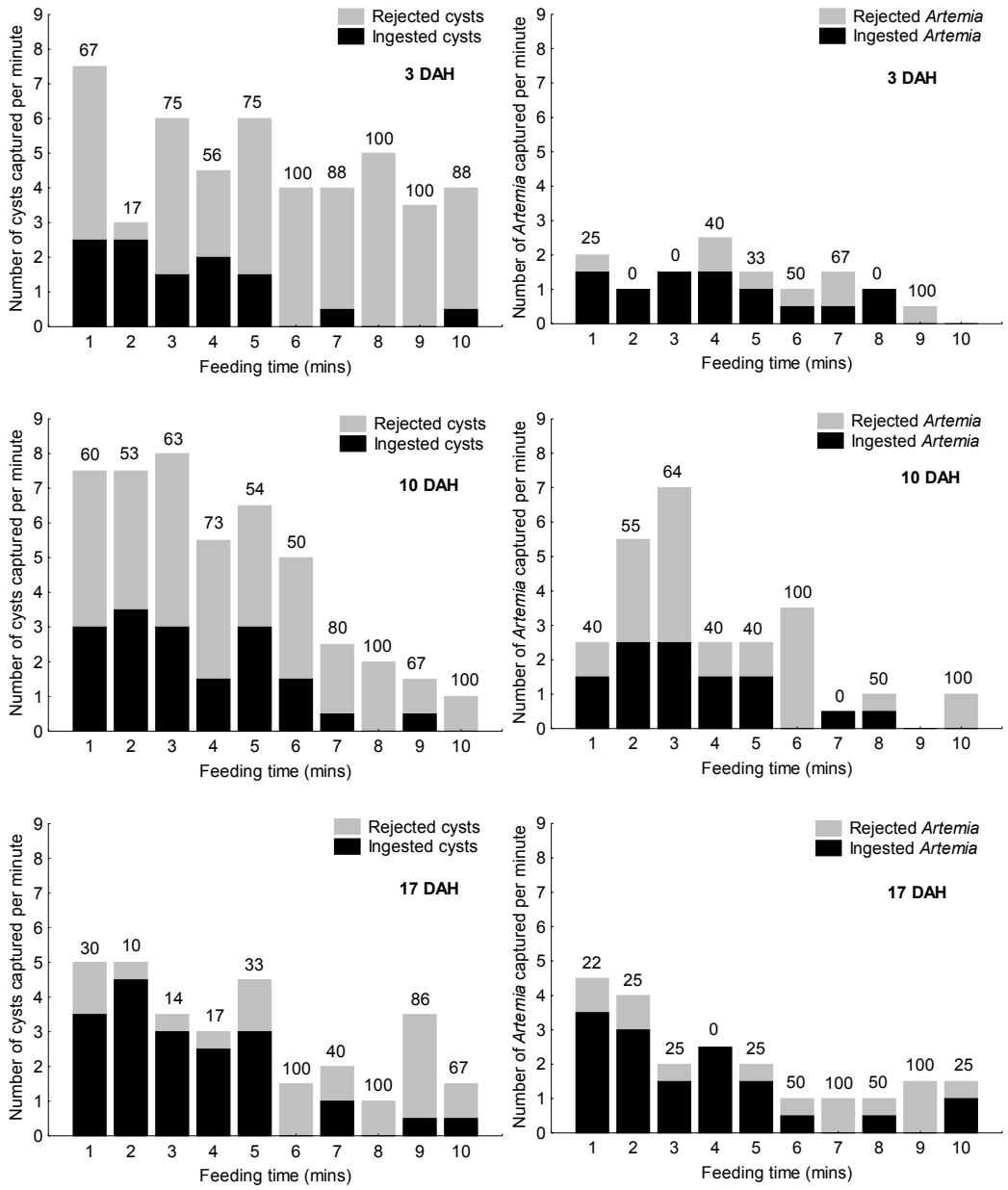


Figure 7.5 Comparison of rejected and ingested *Artemia* cysts and *Artemia* nauplii during the first 10 minutes of feeding at 3, 10 and 17 DAH. Values on top of each histogram represent percentage rejection of the food item at that time.

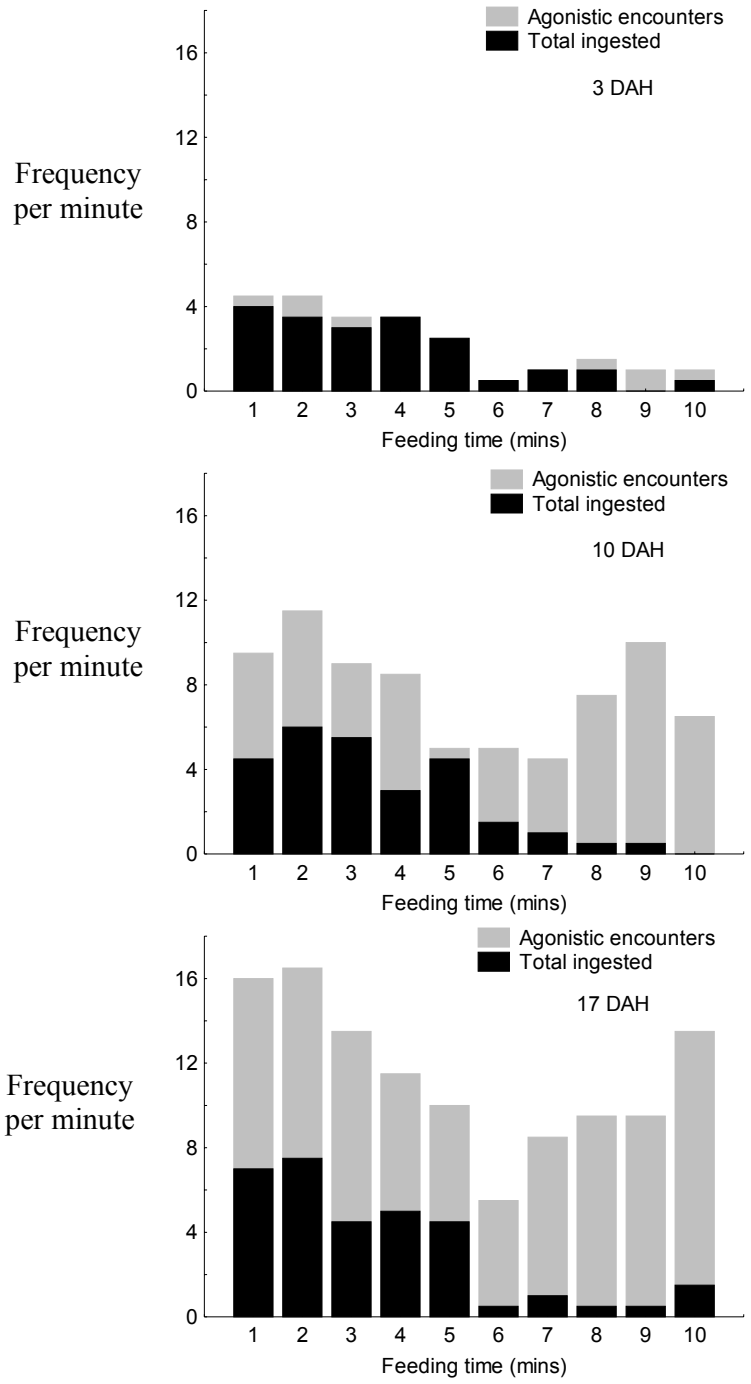
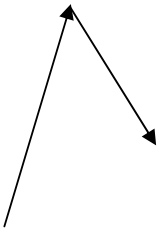
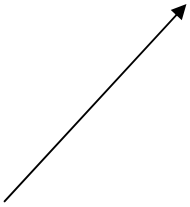
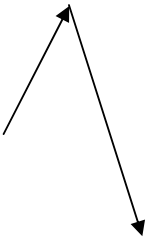
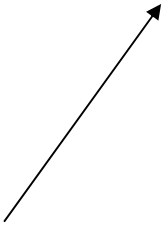
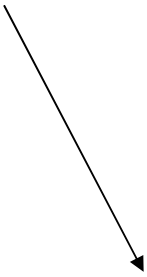
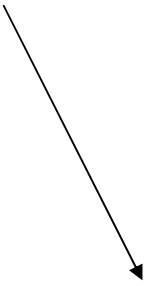


Figure 7.6 Comparison of the number of ingested food items with the frequency of agonistic encounters at 3, 10 and 17 DAH during the first 10 minutes of feeding.

Discussion

Goldfish larvae reached satiation after five minutes in the time period from 3 – 17 DAH. The frequency of agonistic encounters was not related to satiation (Table 7.3). As fish grew they ingested a greater number of prey items and the frequency of agonistic behaviour increased. At 17 DAH goldfish juveniles ingested a greater portion of captured prey items than any of the other two days.

Table 7.3 Total number of prey items captured, ingested and rejected and the frequency of agonistic encounters at 3, 10 and 17 DAH for 1 – 5 and 6 – 10 minutes of feeding.

	# prey items captured	# prey items ingested	% rejection	Frequency of agonistic encounters
1 – 5 minutes				
6 – 10 minutes				
DAH	3 10 17	3 10 17	3 10 17	3 10 17

Goldfish larvae preferred to capture *Artemia* cysts although they rejected a higher percentage of cysts than *Artemia* nauplii. *Artemia* nauplii may have been harder to catch due to their movement in the water column. Cysts may have been more attractive to larvae due to their smaller size (200 to 250 μm) in comparison to *Artemia* nauplii (470 to 550μm) (Verreth *et al*., 1987). The relatively high rejection rate *Artemia* cysts may have been a result of them being less palatable than *Artemia* nauplii. Cyprinids, including goldfish, have highly developed taste buds which play a role in ingestion (Lamb, 2001). Goldfish use chemical cues during intra-oral sorting and rejection/ingestion behaviour (Lamb and Finger, 1995). It is hypothesised that this mechanism becomes more efficient during early development. Thus, more cysts were ingested as fish grew and taste buds developed. To test this hypothesis it would be necessary to study the development of taste buds. The importance of palatability for food preference has been demonstrated in larval sturgeon *A. oxyrinchus desotoi* where feed consumption was greatly enhanced through the addition of a naturally occurring chemical attractant found in the fish's preferred diet (Bardi *et al*., 1998).

Mills *et al*. (1996) reported that goldfish larvae relied on proteolytic enzymes in their food for digestion, and as their digestive system developed, nutrient utilisation improved. This was demonstrated by Vanhaecke *et al*. (1990) who found that when feeding decapsulated cysts to carp larvae, the quantity of cysts could be reduced by up to 35 % after two weeks. Fish zymogens are activated by invertebrate enzymes (Dabrowski, 1991; Abi-Ayad & Kestemont, 1994; Mischke & Morris, 1998). Furthermore, relatively low levels of digestive enzymes in first-feeding larvae could be supplemented by exogenous enzymes from *Artemia* nauplii and cysts (Merchie cited in Lavens & Sorgeloos, 1996).

Capture success in fish larvae was low at first feeding but rose rapidly during early development (Houde & Schekter, 1980). This has been shown to be dependent on larval age and size (Blaxter, 1986), improved manoeuvrability (Hurst, 1994), and feeding experience (Colgan *et al*., 1986), all of which improved over time. Atlantic cod larvae, *G. morhua*, attacked any prey encountered for the first few weeks of feeding but capture success was initially low and increased as fish become more selective (Puvanendran & Brown, 1999).

In African catfish *C. gariepinus*, aggressive behaviour amongst larvae was more frequent during feeding than during resting and swimming (Hecht *et al*., 1996). However, larval and juvenile goldfish were equally aggressive before and after satiation. Agonistic behaviour increased significantly after metamorphosis at 10 DAH which was similar to Japanese flounder *Paralichthys olivaceus* whereby agonistic behaviour began after the completion of metamorphosis (Sakakura & Tsukamoto, 2002). It is hypothesised that an increase in swimming ability increased the frequency of contact between fish.

In summary, Chapters 4, 5 and 6 showed that goldfish larvae grew best on decapsulated cysts. Results of this study showed that they captured more cysts than nauplii on each of the days tested. At 17 DAH juveniles ingested more cysts than they rejected. Thus, it is hypothesised that cysts were easier to catch or were more attractive to larvae due to their smaller size, but were less palatable to younger fish. *Artemia* nauplii were more difficult to capture but appeared to be more palatable to larval goldfish. At 17 DAH, fish ingested more decapsulated cysts than *Artemia* nauplii and rejection of cysts was low. Agonistic behaviour during feeding was not affected by satiation and increased after the completion of metamorphosis.

Chapter 8

General Discussion

This study was designed to describe morphometric development in larval and juvenile goldfish fed different diets, i.e. dry food, *Artemia* nauplii and decapsulated *Artemia* cysts. By measuring various morphometric values the study contributed to an understanding of factors modifying growth and development (Figure 8.1). Using this knowledge, recommendations could be made regarding the use of decapsulated cysts and the relationship between feeding intensity and growth. Finally, a behavioural observation helped to understand the effect of food preference on growth.

This study demonstrated that decapsulated *Artemia* cysts are a good alternative to live *Artemia* nauplii for rearing goldfish larvae and juveniles. Growth and survival was higher in fish fed cysts than in those fed *Artemia* nauplii or a mixed diet of dry food and nauplii (Figure 4.2). In addition, cysts remain available to the fish for a longer period, produce better growth, and are easier to prepare and feed (Kaiser *et al*., 2003). Reasons for this improved growth include: instar I *Artemia* nauplii have a lower energy content because they do not feed and consume their energy reserves (Dhert *et al*., 1997); and the behaviour observations suggested that goldfish juveniles preferred cysts to nauplii (Chapter 7). Studies involving the use of decapsulated cysts indicated their suitability as a diet for other cyprinid species such as the grass carp larvae, *Ctenopharyngodon idella*, which grew better when fed cysts rather than nauplii (Jähnichen & Kohlmann, 1999). Larval development, growth and survival, and size variation were influenced by feeding cysts even under a range of conditions such as feeding level and different tank designs.

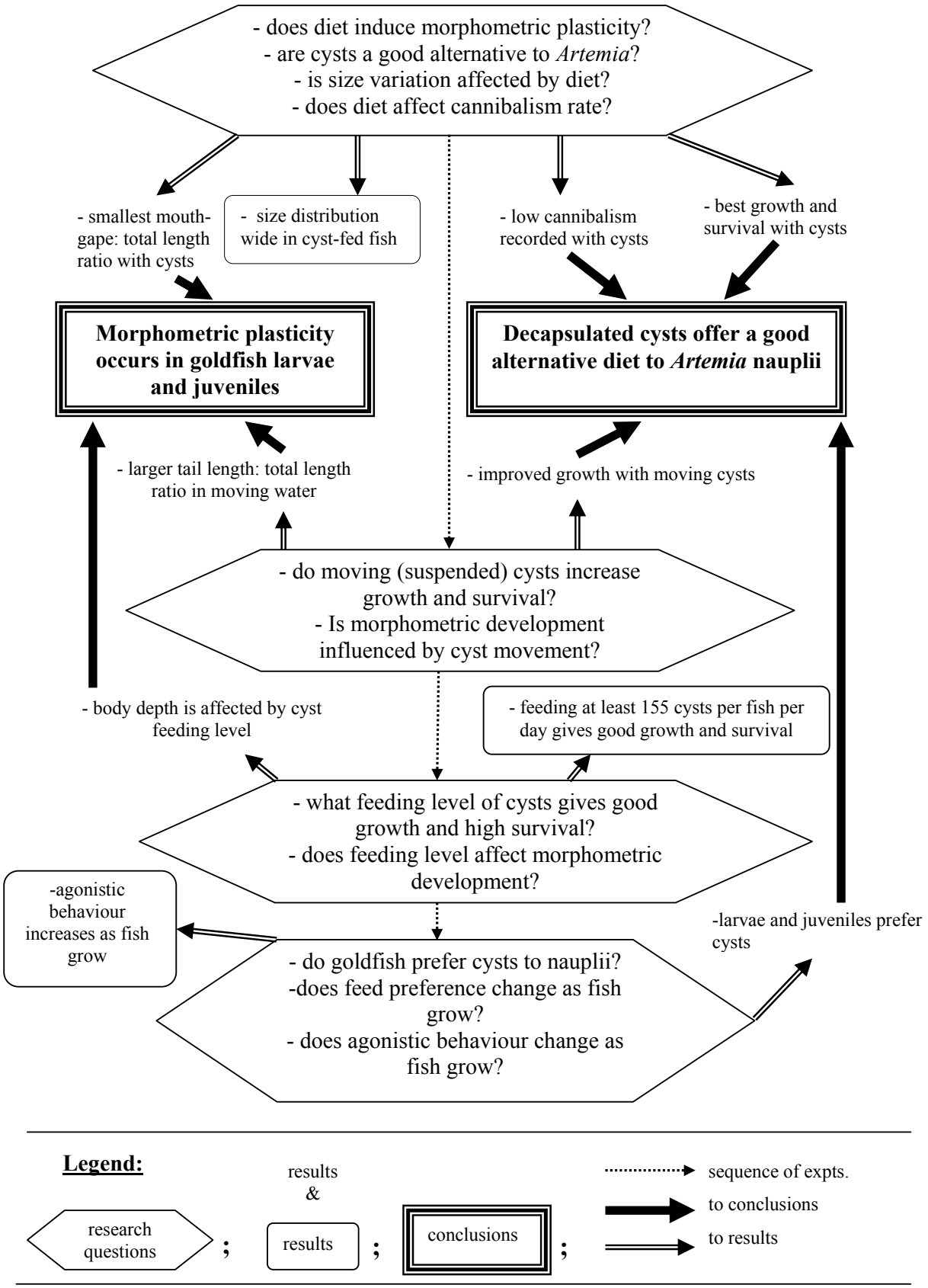


Figure 8.1 Project design showing main objectives, results and conclusions.

Larval development

The timing of metamorphosis in goldfish was affected by feeding (Figure 8.2) and morphometric plasticity was exhibited within two weeks of growth. For example, fish fed cysts completed metamorphosis ten days after hatching while this was delayed by one day in larvae fed *Artemia* nauplii and in fish fed less than 93 cysts per fish per day. Diet-induced phenotypic plasticity occurred in the cichlid, *Cichlasoma managuense* (Meyer, 1987), orange-spotted sunfish, *Lepomis humilis* (Hegrenes, 2001), sticklebacks, *Gasterosteus aculeatus* (Schluter, 1993; 1995), Arctic charr, *Salvelinus alpinus* (Magnusson & Ferguson, 1987), and a *Barbus* species (Nagelkerke, 1997). In these studies, fish were measured after a couple of weeks or months. This research indicated that it is possible to determine the effect of diet type on development as early as fourteen days after hatching.

Mouth-gape in relation to head depth and total length differed according to environmental conditions such as diet type and food presentation but was not affected by feeding level. Fish fed cysts had the smallest mouth-gape to total length ratio compared to fish fed *Artemia* nauplii or a mixed diet. However, fish that picked moving cysts from the water column developed a larger mouth in relation to head depth and total length (Chapter 5). This suggested an adaptation to different environmental conditions to improve feeding success. Cysts moving in up-welling water (Chapter 5) or swimming *Artemia* nauplii needed to be hunted. The adaptation may have led to a conservation of energy, and coupled with the higher nutritional value of *Artemia* cysts, this may explain the relatively larger total length of larvae fed cysts compared to those fed nauplii and the mixed diet. It is hypothesised that goldfish invest relatively more energy in length than mouth development when they are able to consume a high quality diet that requires little effort to search for. This should be tested in future studies.

Body depth in proportion to total length was used to judge fish condition. It differed with feeding level but not with environmental conditions. With an increase in feeding level of cysts, body depth increased in proportion to total length. At 16 DAH, fish fed the same number of cysts in flat-bottomed as those in V-shaped tanks were in similar condition while being smaller in size (Chapter 5). Goldfish did not effectively browse cysts off the bottom of flat-bottomed tanks which may explain why they were smaller than those from V-shaped tanks. However, this method of feeding allowed them to maintain a similar body shape to those that captured moving cysts. Fish in V-shaped tanks had a relatively larger mouth-gape which leads to the hypothesis that the potential for morphometric variation in mouth-gape is an adaptation to feeding strategy rather than a result of nutritional differences.

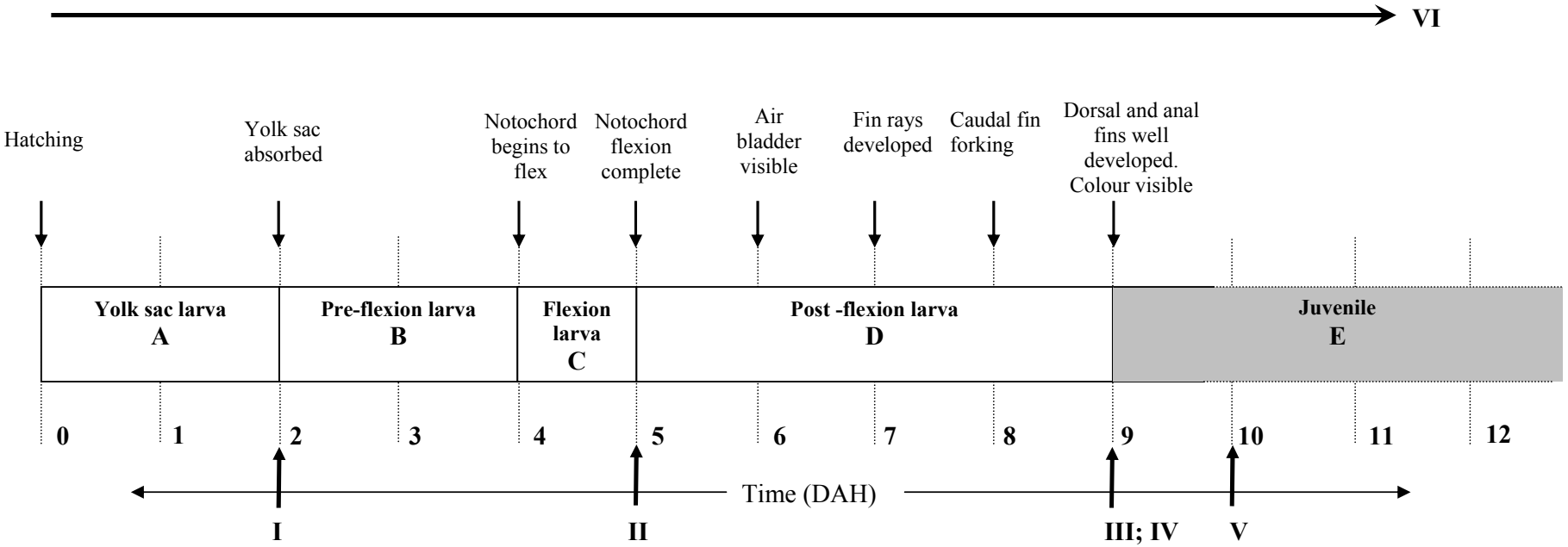
Overall, diet-type induced changes in development. This may have implications for the control of cannibalism as feeding decapsulated cysts and manipulation of the rearing environment reduced mouth-gape. Studies should be designed to test these predictions in other fish species with a wide range of feeding habits.

Larval growth and survival

Goldfish larvae fed 93 decapsulated *Artemia* cysts per fish per day grew better than those fed that number of *Artemia* nauplii. However, at this feeding rate in flat-bottomed tanks size variation was highest in fish fed cysts. Experiments for Chapters 5 and 6 were designed to reduce size variation in fish fed decapsulated cysts and to improve growth and survival. One possible explanation for the larger size variation in cyst-fed fish was that some fish were ineffective at browsing cysts from the bottom of the tank. Failure to browse decapsulated cysts from the bottom of culture tanks has been described in larval carp *C. carpio* where poor growth and a wide size distribution was found (Bryant & Matty, 1980).

In V-shaped tanks inflowing water was directed upwards from the tank bottom keeping the cysts moving in the water column. This lead to a lower size variation and improved growth of the larvae compared with flat-bottomed tanks (Chapter 5). Under-feeding has been shown to increase size variation among fish larvae (Wankowski & Thorpe, 1979; Hecht & Pienaar, 1993; Abi-Ayad & Kestemont, 1994; and Goldan *et al*., 1998). Therefore, a study was conducted to determine the feeding level that would be best for growth and survival in goldfish juveniles while reducing size variation (Chapter 6). Results from this experiment showed that feeding cysts at 155 cysts per fish per day gave the best growth and survival after 16 DAH. As no maximum growth was found, better growth might have been possible at a feeding level greater than 155 cysts per fish per day.

Survival of cyst-fed goldfish larvae was high (98%) at feeding levels of at least 93 cysts per fish per day. Good survival has been reported for other cyst-fed cyprinid larvae such as common carp *C. carpio* (Jähnichen & Kohlmann, 1999) and grass carp *C. idella* (Vanhaecke *et al.,* 1990).



Key:
I = initiation of exogenous feeding;
II = Metamorphosis completed; cannibalism first observed in fish fed *Artemia* nauplii;
III = peak in mouth-gape: total length ratio;
IV = peak in mouth-gape: head depth ratio;

BEHAVIOUR
V = peak in prey capture and prey rejection
VI = increase in agonistic behaviour

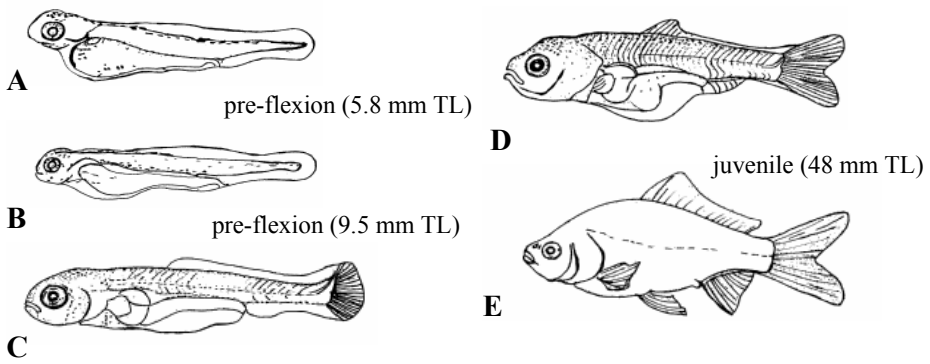


Figure 8.2 Timeline of larval and juvenile goldfish development for fish fed decapsulated *Artemia* cysts indicating time at which metamorphosis was completed. Larval and juvenile drawings adapted from Pinder, (2001).

Behaviour

Behaviour observations were conducted to explain why cysts performed better than nauplii as a diet for larval goldfish. Goldfish larvae and particularly juveniles preferred to ingest cysts and selected more cysts than *Artemia*. Perhaps the high initial rejection rate of cysts in young larvae was due to un-palatability of cysts but the small size and ease of capture may have provided stronger cues for the larvae to ingest. Goldfish rely on highly developed taste buds for feeding (Lamb, 2001) and it is thus possible that young larvae were not able to decide if cysts were edible. Preference for *Artemia* nauplii or cysts was not examined for flat-bottomed tanks. The better growth in fish from flat-bottomed tanks may have reflected the high nutritional value of cysts compared to nauplii. On average, they have 30-40 % higher energy content than *Artemia* nauplii (Vanhaecke *et al*., 1983; 1990).

As with African catfish *Clarias gariepinus* (Hecht & Appelbaum, 1988) and Koi carp *C. carpio* (Van Damme *et al*., 1989), agonistic behaviour in goldfish increased with age. Despite this increase in agonistic behaviour, the incidence of cannibalism in goldfish larvae and juveniles was low. This can be attributed to the relatively small size variation recorded for these experiments. A wide size distribution has been shown to be a catalyst for larval cannibalism (Ruzzante, 1994) and it is hypothesised that feeding an adequate number of cysts to larval goldfish reduced size variation in the population to a level that excluded cannibalism. Chapters 4, 5 and 6 related mouth-gape to total length and head depth under different experimental conditions, and at feeding levels above 93 cysts per fish per day, fish were not able to eat their siblings whole. In commercial culture systems, sibling cannibalism among goldfish larvae may be more problematic when different size classes are reared together but under the conditions described in this thesis, cannibalism was very low.

Conclusions

Time required to complete metamorphosis in goldfish larvae was reduced by increasing the feeding level of cysts (Chapter 6) and by making the cysts more accessible to the fish (Chapter 5). This supports the hypothesis that fish size is a more accurate measure than fish age for determining when larvae completed metamorphosis. This is important because feeding and agonistic behaviour changes after metamorphosis (Chapter 7). From the results of this thesis it is hypothesised that the morphometric plasticity recorded for goldfish mouth-gape is an adaptive response to the rearing environment and not a result of nutritional differences between diets.

Decapsulated cysts were found to have the following advantages over live *Artemia* nauplii and a mixed live/dry diet: (1) goldfish larvae and juveniles grew better on cysts; (2) size variation was lowest when feeding rate was high and cysts were kept off the tank bottom; (3) goldfish larvae and juveniles preferred cysts; (4) there was less fouling of the culture tanks; and (5) cysts last longer in freshwater than nauplii and dry food; (5) cyst-fed fish completed metamorphosis at a younger age which may contribute to a reduction in feed cost in commercial systems.

References

ANONYMOUS. 2000. Goldfish - *Carassius auratus* (Linnaeus). http://elib.cs.berkeley.edu /kopec/tr9/html/sp-goldfish.html .

ABI-AYAD, A. and KESTEMONT, P. 1994. Comparison of the nutritional status of goldfish (*Carassius auratus*) larvae fed with live, mixed or dry diet. *Aquaculture* **128**: 163-176.

ALAMI-DURANTE, H., CHARLON, N., ESCAFFRE, A. M., and BERGOT, P. 1991. Supplementation of artificial diets for common carp (*Cyprinus carpio* L.) larvae. *Aquaculture* **93**: 167-175.

APPELBAUM, S. and ULAND, B. 1979. Intensive rearing of grass carp larvae *Ctenopharyngodon idella* (Valenciennes 1844) under controlled conditions. *Aquaculture* **17**: 175-179.

ARTS, M. and EVANS, D. O. 1987. Precision micrometer measurement of mouth gape of larval fish. *Can. J. Fish. Aquat. Sci.* **44**: 1786-1791.

BAILEY, K. M. and HOUDE, E. D. 1989. Predation on eggs and larvae of marine fishes and the recruitment problem. *Adv. Mar. Biol.* **25**: 1-83.

BARDI, R. W., CHAPMAN, F. A., and BARROWS, F. T. 1998. Feeding trials with hatchery-produced Gulf of Mexico sturgeon larvae. *The Prog. Fish Cult.* **60**: 25-31.

BARTLEY, D. M. 1999. Sustainable use of ornamental fishery resources: Global issues. (abstract). In Conference Abstracts, AQUARAMA 1999. World Conference on Ornamental Fish Aquaculture. 3-6 June 1999, pp.81. Singapore.

BLAXTER, J. H. S. 1986. Development of sense organs and behaviour of teleost larvae with special reference to feeding and predator avoidance. *Trans. Am. Fish. Soc.* **115**: 98-114.

BRYANT, P. L. and MATTY, A. J. 1980. Optimisation of *Artemia* feeding rate for carp larvae (*Cyprinus carpio* L.). *Aquaculture* **21**: 203-212.

BRYANT, P. L. and MATTY, A. J. 1981. Adaptation of carp (*Cyprinus carpio*) larvae to artificial diets. 1. Optimum feeding rate and adaptation age for a commercial diet. *Aquaculture* **23**: 275-286.

ÇALTA, M. 2000. Morphological development and growth of chub, *Leuciscus cephalus* (L.), larvae. *J. Appl. Ichthyol*. **16(2)**: 83-87.

CHAKRABARTI, R. and JANA, B. B. 1991. Plankton intake as a function of body weight by common carp fry under different feeding conditions. *Aquaculture* **93**: 21-34.

CHAPMAN, F. A. and FITZ-COY, S. A. 1997. United States of America trade in ornamental fish. *J. World Aquacult. Soc.* **28(1)**: 1-10.

CHARLON, N. and BERGOT, P. 1984. Rearing system for feeding fish larvae on dry diets. Trial with carp (*Cyprinus carpio* L.) larvae. *Aquaculture* **41**: 1-9.

COLGAN, P. W., BROWN, J. A., and ORSATTI, S. D. 1986. The role of diet and experience in the development of feeding behaviour in largemouth bass, *Micropterus salmoides*. *J. Fish Biol.* **28**: 161-170.

COX, E. S. and PANKHURST, P. M. 2000. Feeding behaviour of greenback flounder larvae, *Rhombosolea tapirina* (Günther) with differing exposure histories to live prey. *Aquaculture* **183**: 285-297

CRUSHING, D. H. and HORWOOD, J. W. 1994. The growth and death of fish larvae. *J. Plank. Res.* **16**: 291-300.

CUNHA, I. and PLANAS, M. 1999. Optimal prey size for early turbot larvae (*Scophthalmus maximus* L.) based on mouth and ingested prey size. *Aquaculture* **175**: 103-110.

DABROWSKI, K. 1991. Dietary requirements for freshwater fish larvae - in search of a common thread. In: Larvi '91 - Fish and crustacean larviculture symposium. Lavens, P., Sorgeloos, P., Jaspers, E., and Ollevier, F. (eds). 11-13.

DABROWSKI, K. and BARDEGA, R. 1984. Mouth size and predicted food size preferences of larvae of three cyprinid fish species. *Aquaculture* **40**: 41-46.

DEGANI, G. 1990. Effect of diet, population density and temperature on growth of larvae and juveniles of *Trichogaster trichopterus* (Bloch & Schneider 1801). *J. Aqua. Trop.* **6**: 135-141.

DHERT, P., LIM, L. C., CANDREVA, P., and SORGELOOS, P. 1997. Possible applications of modern fish larviculture technology to ornamental fish production. *Aquar. Sci. Cons.* **1**: 119-128.

DOI, M., OHNO, A., KOHNO, Y. T., and SINGHAGRAIWAN, T. 1997. Development of feeding ability in red snapper *Lutjanus argentimaculatus* early larvae. *Fisheries Science* **63 (6)**: 845-853.

DROST, M. R. 1987. Relation between aiming and catch success in larval fishes. *Can. J. Fish. Aquat. Sci.* **44**: 304-315.

DOWER, J. F., PEPIN, P., and LEGGETT, W. C. 1998. Enhanced gut fullness and an apparent shift in size selectivity by radiated shanny (*Ulvaria subbifurcata*) larvae in response to increased turbulence. *Can. J. Fish. Aquat. Sci.* **55**: 128-142.

ELDRIDGE, M. B., WHIPPLE, J. A., ENG, D., BOWERS, M. J., and JARVIS, B. M. 1981. Effects of food and feeding factors on laboratory-reared striped bass larvae. *Trans. Am. Fish. Soc.* **110**: 111-120.

EMLEN, J. M. 1966. The role of time and energy in food preference. *Am. Nat.* **100**: 611-617.

FIOGBÉ, E. D. and KESTEMONT, P. 1995. An assessment of the protein and amino acid requirements in goldfish (*Carassius auratus*) larvae. *J. Appl. Ichthyol.* **11**: 282-289.

FOOD AND AGRICULTURAL ORGANISATION OF THE UNITED NATIONS. 1996. Manual on the production and use of live food for aquaculture. LAVENS, P. and SORGELOOS, P. (eds). Laboratory of Aquaculture and *Artemia* Reference Centre. University of Ghent. Belgium. 79-164.

FOWLER, G. M. and SMITH, S. J. 1983. Length changes in silver hake (*Merluccius bilinearis*) larvae: Effects of formalin, ethanol, and freezing. *Can. J. Fish. Aquat. Sci.* **40**: 866-870.

FUKUHARA, O. 1991. Size and age at transformation in red sea bream, *Pagrus major*, reared in the laboratory. *Aquaculture* **95**: 117-124.

GILL, A. B. and HART, P. J. B. 1996. How feeding performance and energy intake change with a small increase in the body size of the three-spined stickleback. *J. Fish Biol.* **48**: 878-890.

GOLDAN, O., POPPER, D., and KARPLUS, I. 1997. Management of size variation in juvenile gilthead sea bream (*Sparus aurata*). 1: Particle size and frequency of feeding dry and live food. *Aquaculture* **152**: 181-190.

GOLDAN, O., POPPER, D., KOLKOVSKI, S., and KARPLUS, I. 1998. Management of size variation in juvenile gilthead sea bream (*Sparus aurata*) 2. Dry food type and live/dry food ratio. *Aquaculture* **165**: 313-320.

GULBRANDSEN, J. 1993. Prey consumption of Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae: selectivity or availability? *Aquaculture* **116**: 158-170.

GUNNES, K. 1976. Effect of size grading young Atlantic salmon (*Salmo salar*) on subsequent growth. *Aquaculture* **9**: 381-386.

HARTMAN, G. F. 1958. Mouth size and food size in young rainbow trout. *Copeia* **(3)**: 233-234.

HATZIATHANASIOU, A., PASPATIS, M., HOUBART, M., KESTEMONT, P., STEFANAKIS, S., and KENTOURI, M. 2002. Survival, growth and feeding in early life stages of European sea bass (*Dicentrarchus labrax*) intensively cultured under different stocking densities. *Aquaculture*. **205**: 89-102.

HAY, D. E. 1982. Fixation shrinkage of herring larvae: effects of salinity, formalin concentration, and other factors. *Can. J. Fish. Aquat. Sci.* **39**: 1138-1143.

HAY, D. E. 1984. Weight loss and change of condition factor during fixation of Pacific herring, *Clupea harengus*, eggs and lavae. *J. Fish Biol.* **25**: 421-433.

HECHT, T. and APPELBAUM, S. 1988. Observations on intra-specific aggression and coeval sibling cannibalism by larval and juvenile *Clarias gariepinus* (Clariidae: Pisces) under controlled conditions. *J. Zool. (London)* **214**: 21-44.

HECHT, T., BATTAGLENE, S., and TALBOT, B. 1996. Effect of larval density and food availability on the behaviour of pre-metamorphosis snapper, *Pagrus auratus* (Sparidae). *Mar. Freshwater Res.* **47**: 223-231.

HECHT, T. and PIENAAR, A. G. 1993. A review of cannibalism and its implications in fish larviculture. *J. World Aquacult. Soc.* **24 (2)**: 246-261.

HEGRENES, S. 2001. Diet-induced phenotypic plasticity of feeding morphology in the orangespotted sunfish, *Lepomis humilis*. *J. Ecol. Freshwater Fish* **10**: 35-42.

HOLM, J. C. 1986. Yolk sac absorption and early food selection in Atlantic salmon feeding on live prey. *Aquaculture*. **54**: 173-183.

HOLT, G. 1993. Feeding larval red drum on micro-particulate diets in a closed recirculating water system. *J. World Aquacult. Soc.* **24**: 225-230.

HOLT, G. J. 1991. Larval fish nutrition and feeding in closed, recirculation culture systems. Larvi '91 - Fish and crustacean larviculture symposium. Lavens, P., Sorgeloos, P., Jaspers, E., and Ollevier, F. (eds). 172-173.

HOUDE, E. D. and SCHEKTER, R. C. 1980. Feeding by marine fish larvae: development and functional responses. *Env. Biol. Fish.* **5**: 315-334.

HURST, S. 1994. Suction prey capture by clownfish larvae (*Amphiprion perideraion*). *Copeia* **(1)**: 242-246.

IRWIN, S., O'HALLORAN, J., and FITZGERALD, R. D. Stocking density, growth and growth variation in juvenile turbot, *Scophthalmus maximus* (Rafinesque). *Aquaculture* **178**: 77-88.

IRWIN, S., NICHARA, U., FITZGERALD, R. D., O'HALLORAN, J., and O'DRISCOLL, E. 1996. Growth in turbot. http://www.adc.org\triGFFGJ.htm.

ITO, T. and SUZUKI, R. 1977. Feeding habits of the cyprinid loach in the early stages. *Bull. Freshwater Res. Lab.* **27**: 85-94.

JAKOBSEN, P. J. and JOHNSEN, G. H. 1987. The influence of predation on horizontal distribution of zooplankton species. *Freshwater Biol.* **17**: 501-507.

JÄHNICHEN, H. and KOHLMANN, K. 1999. Decapsulated *Artemia* cysts - a good alternative to live zooplankton. Fish Farmer **3**. 30-31.

JENNINGS, S. 1991. The effects of capture, net retention and preservation upon lengths of larval and juvenile bass, *Dicentrarchus labrax* (L.). *J. Fish Biol.* **38**: 349-357.

JOBLING, M., COVÈS, D., DAMSGÅRD, B., KRISTIANSEN, H. R., KOSKELA, J., PETURSDOTTIR, T. E., KADRI, S., and GUDMUNDSSON, O. 2001. Techniques for measuring feed intake. In: HOULIHAN, D., BOUJARD, T., and JOBLING, M (eds.). Food intake in fish. Blackwell Science, Oxford, United Kingdom. 49 pp.

JONES, K. A. 1992. Chemical requirements of feeding in rainbow trout, *Oncorhynchus mykiss* (Walbaum); palatability studies on amino acids, amides, alcohols, aldehydes, saccharides, and other compounds. *J. Fish Biol.* **34**: 413-423.

JONES, D. A., KAMARUDIN, M. S., and LE VAY, L. 1993. The potential for replacement of live feeds in larval culture. *J. World Aquacult. Soc.* **24 (2)**: 199-210.

KAISER, H., ENDEMANN, F., and PAULET, T. G. 2003. A comparison of artificial and natural foods and their combinations in the rearing of goldfish, *Carassius auratus* (L). *Aquacult. Res.* In print.

KESTEMONT, P. 1995. Influence of feed supply, temperature and body size on the growth of goldfish *Carassius auratus* larvae. *Aquaculture* **136**: 341-349.

KESTEMONT, P. and BARAS, E. Environmental factors and feed intake: mechanisms and interactions. In: HOULIHAN, D., BOUJARD, T., and JOBLING, M. (eds.). Food intake in fish. Blackwell Science, Oxford, United Kingdom. 131 pp.

KESTEMONT, P. and MÉLARD, C. 1991. Quantitative feed requirements of goldfish *Carassius auratus* larvae fed with a mixed diet. Larvi '91 - Fish and crustacean larviculture symposium. Lavens, P., Sorgeloos, P., Jaspers, E., and Ollevier, F. (eds.).157-159.

KESTEMONT, P. and STALMANS, J. M. 1992. Initial feeding of European minnow larvae, *Phoxinus phoxinus* L. 1. Influence of diet and feeding level. *Aquaculture* **104**: 327-340.

KHADKAR, R. B. and RAMAKRISHNA RAO, T. 1986. Prey size selection by common carp (*Cyprinus carpio* var. *communis*) larvae in relation to age and prey density. *Aquaculture* **(89)**: 96 pp

KISLALIOGLU, M. 1976. Prey handling time and its importance in food selection by the 15-spined stickleback, *Spinachia spinachia* L. *J. Exp. Mar. Biol. Ecol.* **25**: 151-158.

KOSLOW, J. A., BRAULT, S., FOIRNIER, R. O., and HUGHES, P. 1985. Condition of larval cod (*Gadus morhua*) off southwest Nova Scotia in 1983 in relation to plankton abundance and temperature. *Mar. Biol.* **86 (113)**: 121 pp.

KRUSE, G. H. and DALLEY, E. L. 1990. Length changes in capelin, *Mallotus villosus* (Müller), larvae due to preservation in formalin and anhydrous alcohol. *J. Fish Biol.* **36**: 619-621.

LAMB, C. F. 2001. Gustation and feeding behaviour. In: HOULIHAN, D., BOUJARD, T., and JOBLING, M. (eds.). Food intake in fish. Blackwell Science, Oxford, United Kingdom. 108-112.

LAMB, C. F. and FINGER, T. E. 1995. Gustatory control of feeding behaviour in goldfish. *Physiol. Behav.* **57**: 483-488.

LANDRY, F., MILLER, T. J., and LEGGETT, W. C. 1995. The effects of small-scale turbulence on ingestion rate of fathead minnow (*Pimephales promelas*) larvae. *Can. J. Fish. Aquat. Sci.* **52(8)**: 1714-1719.

LEWIS, D. M. and PEDLEY, T. J. 2001. The influence of turbulence on plankton predation strategies. *J. Theor. Biol.* **210(3)**: 347-365.

LIM, L. C. 2001. Improved feeding and quality control for the ornamental fish industry in Singapore. PhD Thesis. Ghent University, Belgium.

LOCHMANN, R. and PHILLIPS, H. 1996. Nutrition and feeding of baitfish. *Aquaculture Magazine* **3**: 87-89.

LØKKEBORG, S., SKAJAA, K., and FERNO, A. 2000. Food-search strategy in ling (*Molva molva* L.): crepuscular activity and use of space. *J. Exp. Mar. Biol. Ecol.* **247**: 195-208.

MACINTOSH, D. J. and DE SILVA, S. S. 1984. The influence of stocking density and food ration on fry survival and growth in *Oreochromis mossambicus* and *O. niloticus* female X *O. aureus* male hybrids reared in a closed recirculated system. *Aquaculture* **41**: 345-358.

MACKENZIE, B. R. and KIORBOE, T. 1995. Encounter rates and swimming behaviour of pause-travel and cruise larval fish predators in calm and turbulent laboratory environments. *Limnol. Oceanogr.* **40(7)**: 1278-1289.

MAGNUSON, J. J. 1962. An analysis of aggressive behaviour, growth, and competition for food and space in medaka (*Oryzias latipes*). *Can. J. Zool.* **40**: 313-363.

MAGNUSSON, K.P. and FERGUSON, M. M. 1987. Genetic analysis of four sympatric morphs of the Arctic charr, *Salvelinus alpinus* from Thingvallavatn, Iceland. *Env. Biol. Fish.* **20**: 67-73.

MERCHIE, G., LAVENS, P., DHERT, P., GOMEZ, M. G. U., NELIS, H., DE LEENHEER, A., and SORGELOOS, P. 1996. Dietary ascorbic acid requirements during the hatchery production of turbot larvae. *J. Fish Biol.* **49(4)**: 573-583.

MEGREY, B. A. and HINCKLEY, S. 2001. Effect of turbulence on feeding of larval fishes: a sensitivity analysis using an individual-based model. *J. Mar. Sci.* **58(5)**: 1015-1029.

MEYER, A. 1987. Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution* **41:** 1357-1367.

MEYER, A. 1990. Ecological and evolutionary consequences of the trophic polymorphism in *Cichlasoma citrinellum* (Pisces, Cichlidae). *Biol. J. Linn. Soc.* **39**: 279-299.

MILLER, T. J., CROWDER, L. B., RICE, J. A., and BINKOWSKI, F. P. 1992. Body size and ontogeny of the functional response in fishes. *Can .J. Fish. Aquat. Sci.* **49**: 805-812.

MILLS, D., STONE, D., NEWTON, P., and WILLIS, S. 1996. A comparison of three diets for larval goldfish rearing. *Aust. Aquacult.* **7 (1)**: 48-49.

MILLS, E. L., CONFER, J. L., and KRETCHMER, D. W. 1986. Zooplankton selection by young yellow perch: the influence of light, prey density, and predator size. *Trans. Am. Fish. Soc.* **115**: 716-725.

MINKOFF, G. and VILLANI, P. 1990. Mediterranean aquaculture pp. 100-121 In *Optimisation of larval culture methods,* R. FLOS, L. TORT, and P. TORRES (eds.), Ellis Horwood, New York.

MISCHKE, C. C. and MORRIS, J. E. 1998. Growth and survival of larval bluegills in the laboratory under different feeding regimes. *Prog. Fish. Cult.* **60**: 206-213.

MOOKERJI, N. and RAO, T. R. 1993. Patterns of prey selection in rohu (*Labeo rohita*) and singhi (*Heteropneustes fossilis*) larvae under light and dark conditions. *Aquaculture* **118**: 85-104.

MOYLE, P. B. 1976. Inland fishes of California. University of California Press, Berkeley. 405 pp.

MUNK, P. and KIØRBOE, T. 1986. Feeding and growth of larval herring, *Clupea harengus*, in relation to density of copepod nauplii. *Env. Biol. Fish.* **17**: 133-139.

NAGELKERKE, L. A. J. 1997. The barbs of Lake Tana, Ethiopia: morphological diversity and its implications for taxonomy, trophic resource partitioning, and fisheries. PhD Thesis. Agricultural University, Wageningen Institute of Animal Sciences (WIAS), The Netherlands. 12-111.

NAGELKERKE, L. A. J. and SIBBING, F. A. 1996. Efficiency of feeding on zebra mussel (*Dreissena polymorpha*) by common bream (*Abramis brama*), white bream (*Blicca bjoerkna*), and roach (*Rutilus rutilus*): the effects of morphology and behaviour. *Can. J. Fish. Aquat. Sci.* **53(12)**: 2847-2861.

NAKAMURA, N. and KASAHARA, S. 1955. A study on the phenomenon of the tobi-koi or shoot carp. 1. On the earliest stage at which shoot carp appears. *Bull. Jpn. Soc. Sci. Fish.* **21**: 73-76.

OPUSZYNSKI, K. K. and SHIREMAN, J. V. 1993. Strategies and tactics for larval culture of commercially important carp pp. 189-219 In *Strategies and tactics for management of fertilised hatchery ponds,* K. K. OPUSZYNSKI and J. V. SHIREMAN (eds.), Haworth Press Inc., New York.

OSSE, J. W. N., VAN DEN BOOGAART, J. G. M., VAN SNIK, G. M. J, and VAN DER SLUYS, L. 1997. Priorities during early growth of fish larvae. *Aquaculture* **155**: 249-258.

OTT, L. 1988. An introduction to statistical methods and data analysis, 3rd Ed. JEPPES, F (ed.). Merrell Dow Research Institute. Kent Publishing Co., Boston. Page 293.

PINDER, A. C. 2001. Keys to larval and juvenile stages of coarse fishes from the fresh waters in the British Isles. *Freshwater Biol. Assoc. Scientific Publication No.* **60**: 136 pp.

PUVANENDRAN, V. and BROWN, J. A. 1999. Foraging, growth and survival of Atlantic cod larvae reared in different prey concentrations. *Aquaculture* **175**: 77-92.

REZNICK, D. and YANG, T. 1993. The influence of fluctuating resources on life history: patterns of allocation and plasticity in female guppies. *Ecology*. **74**: 2011-2019.

REZNICK, D. N. 1990. Plasticity in age and size at maturity in male guppies (*Poecilia reticulata*): an experimental evaluation of alternative models of development. *J. Evol. Biol.* **3**: 185-203

RADTKE, R. L. and WAIWOOD, K. G. 1980. Otolith formation and body shrinkage due to fixation in larval cod (*Gadus morhua*). *Can. Tech. Rep. Fish. Aquat. Sci.***19**: 929 pp.

RINGLER, N. H. 1979. Selective predation by drift-feeding brown trout (*Salmo trutta*). *J. Fish. Res. Board Can.* **26**: 392-403.

ROTTMANN, R. W., SHIREMAN, J. V., and LINCOLN, E. P. 1991. Comparison of three live foods and two dry diets for intensive culture of grass carp larvae. *Aquaculture* **96**: 269-280.

RUBINSTEIN, D. I. 1981. Individual variation and competition in the Everglades pygmy sunfish. *J. Anim. Ecol.* **50**: 337-350.

RUZZANTE, D. E. 1994. Domestication effects on aggressive and schooling behaviour in fish. *Aquaculture* **120**: 1-24.

SAKAKURA, Y. and TSUKAMOTO, K. 2002. Onset and development of aggressive behaviour in the early life stage of Japanese flounder. *Fish. Sci.* **4**: 854-871.

SANTIAGO, C. B., ALDABA, M. B., and REYES, O. S. 1987. Influence of feeding rate and diet form on growth and survival of Nile tilapia (*Oreochromis niloticus*) fry. *Aquaculture* **64**: 277-282.

SCHAEL, M., RUDSTAM, L. G., and POST, J. R. 1991. Gape limitation and prey selection in larval yellow perch (*Perca flavescens*), freshwater drum (*Aplodinotus grunniens*), and black crappie (*Pomoxis nigromaculatus*). *Can .J. Fish. Aquat. Sci.* **48**: 1919-1925.

SCHLUTER, D. 1993. Adaptive radiation in sticklebacks: size, shape, and habitat use efficiency. *Ecology* **74**: 699-709.

SCHLUTER, D. 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. *Ecology* **76**: 82-90.

SCOTT, W. B. and CROSSMAN, E. J. 1973. Freshwater fishes of Canada. *Bull. Fish. Res. Board Can.* **184**: 966-977.

SHARMA, J. G. and CHAKRABARTI, R. 1999. Larval rearing of common carp *Cyprinus carpio*: A comparison between natural and artificial diets under three stocking densities. J. *World Aquacult. Soc.* **30 (4)**: 490-495.

SHIM, K. F. and BAJRAI, J. R. 1982. Growth rates and food conversion in young guppy (*Poecilia reticulata* Peters) fed on artificial and natural foods. *Singapore J. Pri. Ind.* **10 (1)**: 26-38.

SHIROTA, A. 1970. Studies on the mouth size of fish larvae. *Bull. Jpn. Soc. Sci. Fish.* **36**: 353-368.

SORGELOOS, P., LAVENS, P., and TACKERT, W. 1991. State of the art in larviculture of fish and shellfish. Larvi '91 In: Fish and crustacean larviculture symposium 1991. Lavens, P., Sorgeloos, P., Jaspers, E., and Ollevier, F. (eds.). 3-5.

STONE, N., PARK, E., DORMAN, L., and THOMFORDE, H. 1995. Arkansas Baitfish manual. ARCHER, D. and MAYHUGH, G. (eds.). North Central Association of Colleges and Schools Commission on Institutions of Higher Education, Chicago, Illinois. 1- 45.

TAKIZAWA, K., FUJITA, Y., OGUSHI, Y., and MATSUNO, S. 1994. Relative change in body length and weight in several fish larvae due to formalin fixation and preservation. *Fish. Sci.* **60(4)**: 355-359.

TANDLER, A. and MASON, C. 1983. Light and food density effects on growth and survival of larval gilthead seabream (*Sparus aurata*, Linnaeus; Sparidae). *Israel Oceanographic and Limnological Research,* Collected reprints. Volume **9**: 103-116.

TAYLOR, K. W. and FREEBERG, M. H. 1984. Effect of food abundance on larval lake whitefish, *Coregonus clupeaformis* Mitchill, growth and survival. *Mar. Biol.* **25**: 733-741.

TLUSTY, M. 2002. The benefits and risks of aquacultural production for the aquarium trade. *Aquaculture* **205**: 203-219.

TUCKER, J. W. and CHESTER, A. J. 1984. Effects of salinity, formalin concentration and buffer on quality of preservation of southern flounder (*Paralichthys lethostigma*) larvae. *Copeia* **5**: 981-988.

UMINO, T., ARAI, K., and NAKAGAWA, H. 1997. Growth performance in clonal crucian carp, *Carassius langsdorfii*. Effects of genetic difference and feeding history. *Aquaculture* **155**: 271-283.

VAN DAMME, P., APPELBAUM, S., and HECHT, T. 1989. Sibling cannibalism in koi carp, *Cyprinus carpio* L., larvae and juveniles reared under controlled conditions. *J. Fish Biol.* **35 (Suppl. A)**: 855-863.

VAN STAPPEN, G. 1996. Introduction, biology and ecology of *Artemia*. In: FOOD AND AGRICULTURAL ORGANISATION OF THE UNITED NATIONS. 1996. Manual on the production and use of live food for aquaculture. LAVENS, P. and SORGELOOS, P. (eds.). Laboratory of Aquaculture and *Artemia* Reference Centre. University of Ghent. Belgium. 79-123.

VANHAECKE, P., DE VRIEZE, L., TACKERT, T., and SORGELOOS, P. 1990. The use of decapsulated cysts of the brine shrimp *Artemia* as direct food for carp *Cyprinus carpio* L. larvae. J. *World Aquacult. Soc.* **21 (4)**: 257-262.

VANHAECKE, P., LAVENS, P., and SORGELOOS, P. 1983. International study on *Artemia*. XVIII. Energy consumption in cysts and early larval stages of various geographical strains of *Artemia*. *Annales de la Societé Royale Zoologique de Belgique* **113 (2)**: 155-164.

VANHAECKE, P. and SORGELOOS, P. 1983. International study on *Artemia*. XXX. Bio-economic evaluation of the nutritional value for carp (*Cyprinus carpio* L.) larvae of nine *Artemia* strains. *Aquaculture* **32**: 285-293.

VERRETH, J. and DEN BIEMAN, H. 1987. Quantitative feed requirements of African catfish (*Clarias gariepinus* Burchell) larvae fed with decapsulated cysts of *Artemia*. I. The effect of temperature and feeding level. *Aquaculture* **63**: 251-267.

VERRETH, J., STORCH, V., and SEGNER, H. 1987. A comparative study on the nutritional quality of decapsulated *Artemia* cysts, micro-encapsulated egg diets and enriched dry feeds for *Clarias gariepinus* (Burchell) larvae. *Aquaculture* **63**: 269-282.

VON LUKOWICZ, M. 1976. Experiments on first-feeding of carp fry with alevon and freeze-dried fish. *Bavarian State Institute for Fishery* **156 (5)**: 94-100.

WALLACE, J. C. and KOLBEINSHAVN, A. G. 1988. The effect of size grading on subsequent growth in fingerling Arctic charr, *Salvelinus alpinus* (L.). *Aquaculture* **97**: 100.

WALLACE, J. C., KOLBEINSHAVN, A. G., and REINSNES, T. G. 1988. The effect of stocking density on early growth in Arctic charr, *Salvelinus alpinus* (L.). *Aquaculture* **73**: 101-110.

WANKOWSKI, J. W. J. 1979. Morphological limitations, prey size, selectivity, and growth response of juvenile Atlantic salmon, *Salmo salar*. *J. Fish Biol.* **14**: 89-100.

WANKOWSKI, J. W. J. and THORPE, J. E. 1979. The role of food particle size in the growth of juvenile Atlantic salmon (*Salmo salar* L.). *J. Fish Biol.* **14**: 351-370.

WERNER, R. G. and BLAXTER, J. H. S. 1980. Growth and survival of larval herring (*Clupea harengus*) in relation to prey density. *Can. J. Fish. Aquat. Sci* **37 (7)**: 1063-1069.

WEST-EBERHARD, M. J. 1989. Phenotypic plasticity and the origins of diversity. *Ann. Rev. Ecol. Syst.* **20**: 249-278

WICKINS, J. F. 1985. Growth variability in individually confined elvers, *Anguilla anguilla* (L.). *J. Fish Biol.* **27**: 469-478.

WIEGAND, M. D., BUCHANAN, L. G., LOEWEN, J. M., and HEWITT, C. M. 1988. Effects of rearing temperature on development and survival of embryonic and larval goldfish. *Aquaculture* **71**: 209-222.

WIMBERGER, P. H. 1992. Plasticity of fish body shape. The effects of diet, development, family and age in two species of *Geophagus* (Pisces, Cichlidae). *Biol. J. Linn. Soc.* **45**: 197-218.

WIMBERGER, P. H. 1993. Effects of vitamin C deficiency on body shape and skull osteology in *Geophagus brasiliensis*: implications for interpretations of morphological plasticity. *Copeia* **3**: 342-351.

YIN, M. C. and BLAXTER, J. H. S. 1987. Feeding ability and survival during starvation of marine fish larvae reared in the laboratory. *J. Exp. Mar. Biol. Ecol.* **105**: 73-83.