Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Marte Solli Lindskog

Muscle growth and development in lumpfish (*Cyclopterus lumpus*) larvae in relation to start-feeding diets (*Artemia*, cirripedia, copepod (*Acartia tonsa*) and formulated diet)

Master's thesis in Ocean Resources
Supervisor: Elin Kjørsvik
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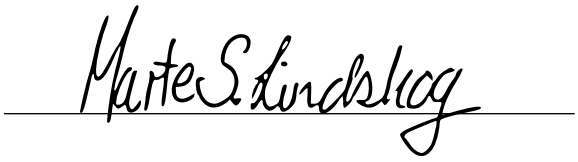
Preface

This master thesis was carried out at the Department of Biology at the Norwegian University of Science and Technology (NTNU). The experiment took place in the laboratories at Sealab NTNU (Trondheim). The experiment was in collaboration with SINTEF Ocean AS and was part of a bigger project called "STARTRENS" (project nr. 901561). The Norwegian Seafood Research Fund (FHF) provided the main funds. The thesis was written under supervision of Professor Elin Kjørsvik and co-supervisor Tu Anh Vo at the department of Biology.

First, I would like to thank my supervisor and co-supervisor for valuable feedback and guidance when writing this thesis and for helping with both the writing and statistics. I would also like to thank the people from SINTEF Ocean AS for good teamwork and discussions. A big thank you for the brilliant guidance in the experimental period; Tu Anh Vo, Luciana Alves Musialak and Frank Thomas Mlingi. Tora Bardal I am very grateful for your assistance and for teaching me the laboratory work. Additionally, thank you to Iurgi Imanol Salaverria-Zabalegui, Arne Kjølsnes and Dag Altin for help with technical problems.

I would also like to thank my fellow students and NJORD at Sealab for an amazing period with coffee breaks, an amazing atmosphere, and interesting discussions. To my two fellow students in this experiment; Sunniva Brevik Kværnø and Saba Akbar I am beyond grateful for the good teamwork and working moral, and lastly our valuable discussions. Finally thank you to family and friends for the great support and the non-stopping encouragements.



Marte Solli Lindskog
Trondheim, november 2021

Abstract

Aquaculture of Atlantic salmon (*salmo salar*) in Norway has been negatively affected by the sea lice (*Lepeophtheirus salmonis*) for a long period. Various chemical therapeutants has been used, but a higher resistance in the sea lice have made farmers look for alternatives. Farming and deployment of cleaner fish which eat the sea lice has been more effective. Today the lumpfish (*Cyclopterus lumpus*) is the most used species, but there are still production problems regarding the larval rearing period. Start-feeding of lumpfish larvae is a problem as the nutritional requirements are still unknown and needs to be identified to be able to obtain the best growth and development in the larvae. Additionally, the lumpfish larvae muscle growth and development are not yet described and can be affected by the diet composition and value.

In the present study a start-feeding experiment was conducted with five different feeding regimes from 2 to 35 dph. Larvae were initially fed either *Artemia*, copepods (*Acartia tonsa*), cirripedia or formulated diet. Additionally, one feeding regime started with copepods first and later changed to cirripedia. All feeding regimes were eventually weaned over to formulated diet. Furthermore, the study aimed to describe the muscle development and growth by histological methods and to evaluate how the different diets affected the development of the muscle, in addition to the general larval growth and survival.

Feeding with *Artemia* provided the best larval growth and survival. The lumpfish muscle had a red fiber layer covering several layers of white muscle fibers at 2 dph. Stratified hyperplasia was present already at 2 dph and seems to have started in the late embryonic stage. Stratified hyperplasia continued until the mosaic hyperplasia pattern was found in all groups on 35 dph. The muscle development was strongly correlated to lumpfish larval size, rather than the larval age. The red muscle grew by hypertrophy first and then by both mechanisms. The white muscle grew by both mechanisms, but the hypertrophy growth was principal.

Sammendrag

Oppdrett av Atlantisk laks (*Salmo salar*) i Norge har vært negativt påvirket av lakselus (*Lepeophtheirus salmonis*) i en lengre periode. Ulike kjemiske midler har blitt brukt for å bli kvitt lakselusen, men en høyere resistans i lakselus mot disse kjemikalene har gjort at oppdrettere har behøvd å sett etter nye alternativer. Oppdrett og utplassering av rensefisk som spiser lakselusen har vist seg å være mer effektivt enn kjemikalier. I dag er det rognkjeks (*Cyclopterus lumpus*) som er den mest brukte rensefiskearten, men det er fortsatt problemer i larveperioden av kultiveringen. Startfôring av rognkjeks er et problem ettersom at næringskravene til rognkjekslarven ikke er kjent og trengs å identifiseres for å oppnå den beste veksten og utviklingen av larven. I tillegg er ikke muskel veksten og utviklingen beskrevet i rognkjeks larver enda.

I denne studien ble det gjennomført et startfôrings forsøk med 5 ulike fôrregimer fra 2 til 35 dager etter klekking. Rognkjekslarvene ble først fôret med enten *Artemia*, copepoder (*Acartia tonsa*), cirripedia eller tørrfôr. I tillegg var det ett fôringsregime som startet med copepoder først før en overgang til cirripedia. Alle fôringsregimene endte til slutt med en tilvenningsperiode over til det samme tørrfôret. Videre vil studien beskrive muskelutviklingen og veksten ved å bruke histologiske metoder og evaluere hvordan de ulike diettene påvirket muskelutviklingen i tillegg til den generelle veksten og overlevelsen i larvene.

Å fôre med *Artemia* ga den beste veksten og overlevelsen i larvene. Rognkjeks-larve muskulaturen hadde et rødt muskellag på utsiden av flere lag med hvit muskel. Stratifisert hyperplasi var til stede allerede to dager etter klekking og startet trolig sent i det embryoniske stadiet. Stratifisert hyperplasi vekst fortsatte til mosaikk hyperplasi vekst ble funnet i alle de ulike fôringsregimene 35 dager etter klekking. Muskelutviklingen var sterkt korrelert til larvestørrelsen, framfor alderen på larvene. Den røde muskelen hadde hypertrofi vekst først, før begge mekanismene var til stede. Den hvite muskelen hadde både hypertrofi og hyperplasi vekst, men hypertrofi veksten var størst.

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Abbreviations

Art	Lumpfish larvae fed *Artemia* for 25 days.
Cir	Lumpfish larvae fed cirripedia for 25 days.
Cop	Lumpfish larvae fed copepods for 16 days.
Cop/Cir	Lumpfish larvae fed copepods for 16 days, and thereafter cirripedia until 25 dph.
DHA	Docosahexaenoic acid (22:6*n*-3) (an essential polyunsaturated omega-3 fatty acid for marine fish larvae).
Dph	Days post hatch (number of days since the lumpfish emerged from its egg).
DW	Dry weight (individual weight of lumpfish dried at 60 °C for 48 hours).
DWI	Daily weight increase (%).
EPA	Eicosapentaenoic acid (20:5*n*-3) (an essential polyunsaturated omega-3 fatty acid for marine fish larvae).
EPAX	Epaxial quadrant of myotome.
FD	Formulated diet
FD-group	Lumpfish larvae fed formulated diet.
FHF	Fiskeri- og havbruksnæringens forskningsfond (Norwegian Seafood Research Fund).
HYPAX	Hypaxial quadrant of myotome.
Ms	Medulla spinalis
NL	Neutral lipid (hydrophobic with no charged groups).
Nt	Notochord
PBS	Phosphate-buffered saline
PFA	Paraformaldehyde
PL	

	Phospholipid (main constitute of cell membrane. A hydrophilic phosphate group and two hydrophobic fatty acids).
R	Red muscle fibers.
SE	Standard error. The standard deviation of the sample population.
SGR	Specific growth rate (the increase in dry weight over a given time interval).
SL	Standard length (measured from the snout to the back of the notochord).
W	White muscle fibers.

1 Introduction

1.1 Lumpfish in aquaculture

1.1.1 Lumpfish as a cleaner fish

The aquaculture industry is characterized as a rapidly growing industry and in 2016 the industry produced more fish for human consumption compared to the amount of fish the fisheries captured (FAO, 2020). Global production of Atlantic salmon (*Salmo salar*) was 2.4 million tonnes in 2018 (FAO, 2020) and Norway is the top producer of Atlantic salmon with approximately 1.3 million tonnes annually (SSB, 2020, FAO, 2020, Grefsrud et al., 2020). Farming of Atlantic salmon in Norway is important because it brings the country large economic incomes with an export value of 72.5 billion NOK in 2019 (Grefsrud et al., 2020, FAO, 2020).

An increased salmon production and a higher density of salmon in the sea cages has increased the prevalence of the ectoparasite sea lice (*Lepeophtheirus salmonis*) (Liu and Bjelland, 2014). The sea lice feeds on the salmon's skin, blood, and mucus (Liu and Bjelland, 2014). Consequently, the salmon becomes more vulnerable for secondary bacterial infections, osmoregulatory failure and death (Liu and Bjelland, 2014). The sea lice impair both the welfare and health of the salmon, together with the economical profit of the farmers (Costello, 2009, Torrissen et al., 2013). The cost of combating sea lice in Norway was estimated to 4.5 billion NOK in 2017 (Nofima, 2017).

Chemical therapeutants and biological control are the treatments commonly used to combat sea lice (Burridge et al., 2010, Grutter, 2010). Chemical therapeutants like hydrogen peroxide and emamectin benzoate among others have caused elevated stress in the salmon and had detrimental effects on the surrounding environment (Aaen et al., 2015). Lately, the sea lice have obtained an increased resistance towards the chemicals (Aaen et al., 2015) and therefore alternative delousing methods are required. This includes the use of biological control with cleaner fish, which feeds on parasites of other fish species (Grutter, 2010). The species used as cleaner fish today are lumpfish (*Cyclopterus lumpus*), ballan wrasse (*Labrus bergylta*), goldsinny wrasse (*Ctenolabrus rupestris*), corkwing wrasse (*Symphodus melops*) and rock-cook (*Centrolabrus exoletus*) (Norwegian Directorate of Fisheries, 2020).

In 2019 in Norway 17.6 million wild caught- and 43.4 million farmed cleaner fish were used to combat sea lice (Figure 1.1) (Norwegian Directorate of Fisheries, 2020). Of the farmed cleaner fish, 42.7 million of them were lumpfish (*Cyclopterus lumpus*) (Norwegian Directorate of Fisheries, 2020). The lumpfish larvae have a considerably higher survival rate (>80%) (Marthinsen et al., 2018, Rian et al., 2019, Dahle et al., 2017, Hanssen et al., 2018) compared to the ballan wrasse (10%) during start-feeding (Berg et al., 2012, Romundstad, 2015, Øie et al., 2015). The lumpfish production cycle is remarkably shorter (4-7 months) compared to the ballan wrasse (1.5 year) and start-feeding of lumpfish is easier than for ballan wrasse (Powell et al., 2018b, Norwegian Seafood

Research Fund, 2014). Lumpfish can continue feeding sea lice down to 4°C (Nytrø et al., 2014) meaning they can potentially survive winters alongside the salmon in the sea cages (Nytrø et al., 2014). This is favorable compared to the wrasse species which are more temperature sensitive and not as effective when the temperature drop below 6°C (Sayer and Reader, 1996). This is an advantage considering the cold temperatures along the Norwegian coastline (Nytrø et al., 2014). Temperature can affect both the metabolism and the locomotory capabilities in the lumpfish (Hvas et al., 2018) as it is a cold-water species native to the east and west boreal region of the North Atlantic (Davenport, 1985, Powell et al., 2018a).

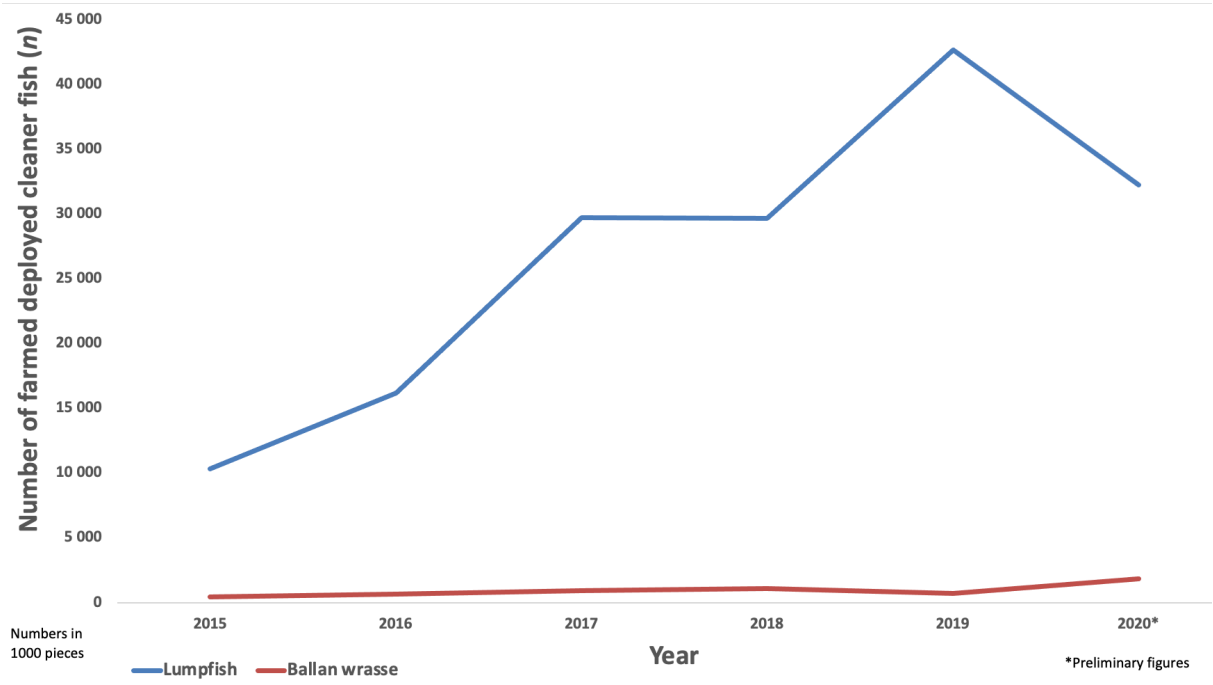


Figure 1.1 Number of deployed farmed lumpfish and ballan wrasse in sea cages of Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) from 2015 to 2020. Numbers retrieved from (Norwegian Directorate of Fisheries, 2021).

1.1.2 Knowledge gaps in lumpfish rearing

Lumpfish is a relatively new species in aquaculture, where the first trials of commercial production started in 2011 (Powell et al., 2018b). The lumpfish larvae hatch from demersal eggs (Davenport, 1985) with an egg diameter of approximately 2.3 mm (Kennedy, 2018, Imsland et al., 2019). The Atlantic salmon hatch from demersal eggs, with a 5-7 mm egg diameter (Davenport, 1985, Kjørsvik et al., 2004). Demersal eggs are larger and have a longer incubation time than the pelagic eggs which ballan wrasse and Atlantic cod hatch from (Kjørsvik et al., 2004, Gagnat et al., 2016). The egg diameter is approximately 1 mm in ballan wrasse and 1.1-1.7 mm in cod (Kjørsvik et al., 2004, Ottesen et al., 2012). The larger demersal eggs contain more yolk and are therefore provided with more energy and are hence better developed at hatching and at start-feeding (Kjørsvik et al., 2004). The lumpfish is hence better developed than the pelagic species, but less than the Atlantic salmon at hatching (Kjørsvik et al., 2004, Gagnat et al., 2016, Davenport, 1985).

Start-feeding of lumpfish larvae in culture is a challenge because the nutritional requirements are still poorly understood. Therefore, requirements of other marine fish larvae in aquaculture have been used as a starting point. Atlantic salmon is fed formulated pellets (Smith et al., 1995), whereas Atlantic cod and ballan wrasse are fed live feeds like *Artemia*, rotifers, copepods, and other natural zooplanktons (Conceição et al., 2010, Gagnat et al., 2016). Thus, both formulated pellets and live preys have been used in start-feeding of lumpfish larvae.

Start-feeding with formulated pellets have led to poor and variable growth and survival in lumpfish, whereas *Artemia* nauplii have led to better growth and survival (Marthinsen et al., 2018, Rian et al., 2019, Hanssen et al., 2018, Belova, 2015, Nytrø et al., 2014). Still, many commercial producers feed their lumpfish larvae exclusively with formulated pellets, since producing live prey is labor intense and expensive (Hamre et al., 2013). Lumpfish larvae hatch at around 5-6 mm SL (Marthinsen et al., 2018, Rian et al., 2019) which is considerably smaller than the Atlantic salmon at 17-20 mm (Kjørsvik et al., 2004) but larger than Atlantic cod and ballan wrasse hatching at around 4-6 mm (Kjørsvik et al., 2004, Berg et al., 2012). This makes it challenging to decide whether to select the Atlantic salmon or the smaller pelagic fish species as a starting point for lumpfish feeding. In smaller fish larvae with a poorly developed digestive system or a not fully differentiated stomach, the digestion of live feed can be easier than formulated diet whereas live preys can be too small for larger fish larvae's (Conceição et al., 2010). The stomach in lumpfish larvae is not fully differentiated until 34 dph, but the formation of stomach gastric glands was observed at 10 dph (Marthinsen et al., 2018). A solution can therefore be to investigate feeding regimes consisting of formulated diet and different live preys and feed compositions, different weaning periods and with a combination of different live preys.

In the wild, the zooplankton copepod is a natural prey organism for several marine fish species, including the lumpfish (Davenport, 1985). Copepod could therefore potentially be a natural prey organism when lumpfish is reared in culture too. As a diet alone or in supplement to rotifers or *Artemia* nauplii, feeding with copepods have improved growth rates and survival in lumpfish, Atlantic halibut, ballan wrasse and Atlantic cod (Dahle et al., 2017, Shields et al., 1999, Øie et al., 2015, Romundstad, 2015, McEvoy et al., 1998). Copepods have a high content of the polyunsaturated fatty acids docosahecaenoic acid (DHA, 22:6*n*-3) (13.9-42.3% of DW) and eicosapentaenoic acid (EPA, 20:5*n*-3) (8.3-24.6% of DW) (Evjemo and Olsen, 1997) located primarily in their phospholipids (Albers et al., 1996). This location provides a more effective utilization for larval growth compared to polyunsaturated fatty acids in neutral lipids (Gisbert et al., 2005, Wold et al., 2007, Kjørsvik et al., 2009, Wold et al., 2009). These two fatty acids cannot be synthesized by marine fish larvae, and are therefore essential to provide in the larval diet (Støttrup, 2003).

Artemia nauplii is not a natural prey organism for cold water fish (Davenport, 1985) but are frequently used in cultivation of marine fish larvae. Enhanced growth rates (standard length, wet and dry weight) and improved survival rates were obtained in lumpfish larvae which received enriched *Artemia* nauplii, compared to copepods (*Acartia tonsa*) and formulated diet (Marthinsen et al., 2018, Hanssen et al., 2018, Rian et al., 2019). *Artemia* nauplii have no DHA and variable amounts of EPA naturally which are essential for marine fish larvae (Conceição et al., 2010, Støttrup, 2003). The reason for their increased use anyhow is because it is possible to enrich the nauplii with DHA and EPA

(Navarro et al., 1999). Additionally, the small size (400-500 μm long) and slow swimming of the newly hatched *Artemia* nauplii makes them suitable as live feed for fish larvae (Øie et al., 2011). Easy handling and availability and the *Artemia*'s ability to be stored for longer periods are advantages, in addition to the relatively high rearing densities which can be obtained (Øie et al., 2011).

Cirripedia nauplii is a natural diet for the marine fish larvae and like the copepods, cirripedia is naturally rich in EPA and DHA in the phospholipids (Tokle, 2021). The Norwegian company Planktonic AS developed the Cryoplankton product consisting of cirripedia nauplii and a cryoprotectant agent (CPA). The eggs are extracted from adult barnacles (Tokle, 2021). Cirripedia nauplii is available in two different sizes and can therefore be used over a longer start-feeding period instead of switching between live preys (Tokle, 2021). Earlier weaning to dry feed and better survival and quality is observed in ballan wrasse juveniles (Tokle, 2021).

Start-feeding with live preys in lumpfish larvae have become a common practice as the formulated diet have resulted in variable growth and survival. The pellets sink faster to the tank bottom and are drifting with the water flow. A common practice have been to provide marine fish larvae with copepods, but in the recent years *Artemia* nauplii have provided better growth and survival in the lumpfish larvae (Marthinsen et al., 2018, Rian et al., 2019, Hanssen et al., 2018, Belova, 2015, Nytrø et al., 2014). The cirripedia nauplii is a relatively new live prey which have been anticipated to be a replacement for the *Artemia* (Tokle, 2021). The copepods and cirripedia have high amounts of EPA and DHA in the phospholipids providing a more efficient utilization compared to the *Artemia*'s location in the neutral lipids (Gisbert et al., 2005, Wold et al., 2007, Kjørsvik et al., 2009, Wold et al., 2009, Tokle, 2021). The feed composition is important for larval growth (Hamre et al., 2013). Finding an optimal feeding regime which enhances larval growth and survival can provide a stable and predictable commercial production for farmers.

1.2 Why study larval lumpfish muscle

Lumpfish is a semi pelagic species, spending much of its adult life offshore, but breed in near-shore waters (Davenport, 1985). However it does not seem to meet the expectation of a fish that is partly of pelagic nature (Hvas et al., 2018) as the lumpfish lacks a swim bladder (Davenport and Kjørsvik, 1986). Consequently it relies heavily on a low density body for buoyancy, i.e the density difference between the surrounding water and the fish (Davenport and Kjørsvik, 1986). Additionally, the lumpfish have a poor maximum swimming speed and a relatively low aerobic scope meaning less aerobic energy is available for activities like locomotion and foraging (Hvas et al., 2018). Even though its morphology points in the direction of a less active swimmer (Davenport and Kjørsvik, 1986), the adult lumpfish naturally migrate annually between feeding and spawning grounds (Davenport, 1985). Swimming is furthermore used to avoid predators and catch food. Already in the embryonic stage the muscle starts to develop, thus investigating how it grows in the larval stage could be interesting.

1.2.1 Muscle fiber types and growth mechanisms

Larval fish muscle is often divided into red and white fibers. Red fibers use aerobic metabolism and contributes to slow swimming behavior such as eating and migrating (Johnston, 1999). White fibers use aerobic metabolism in early larval stage and changes gradually to anaerobic metabolism during metamorphosis (Johnston, 1999). White fibers contribute to fast swimming during hunting or fighting behavior, when high swimming speed is necessary (Johnston, 1999). At hatching, a single layer of red muscle fibers is covering several layers of white muscle fibers and the white muscle fibers constitute approximately 90% of the muscle mass in teleost fish (Johnston, 1999).

The skeletal muscle is the most rapidly growing tissue in fish (Osse and Van den Boogaart, 1995) and grows by two different mechanisms; hyperplasia (increase in fiber number) and hypertrophy (increase in volume of already existing fibers) (Johnston, 1999). Muscle growth in mammals occurs mainly by hypertrophy of already existing fibers formed prior to birth by hyperplastic growth (Johnston, 1999). In fish, hypertrophic growth also generally occur at all times (Johnston, 1999). The hyperplastic growth mostly stops at birth in mammals, and this differs from muscle growth in fish. In fish, it can continue throughout the life span (Weatherley et al., 1988). The hyperplastic growth is usually divided into three phases (Johnston, 1999). The first hyperplastic growth phase starts in the embryonic stage, where muscle fibers form in the somite. The second phase starts late in the embryonic stage or after hatching and continue into the larval stages (Rowlerson, 2001, Johnston, 2006). This phase is termed stratified hyperplasia because new white fibers form along discrete germinal zones in a stratified pattern between red and white fibers at the lateral borders of the myotome (Figure 1.2) (Rowlerson, 2001, Johnston, 2006). The last hyperplastic phase normally occurs in early juvenile or adult stages in large fish species (Weatherley et al., 1988, Johnston, 2006) and can continue throughout the fishes lifespan, until they reach 40% of their maximum length (Weatherley et al., 1988) and 60% of their total body mass (Alami-Durante et al., 1997). This phase is called mosaic hyperplasia since new myoblasts form a mosaic of fiber diameters in between muscle fibers over the whole myotome (Johnston, 2006).

When the skeletal muscle growth mechanisms occur, seems to be different between species (Weatherley et al., 1988). In the smaller fish species, which has a relatively small adult size like fish minnow (*pimephales notatus*) and larval guppy, the muscle grew mainly by hypertrophy (Weatherley et al., 1988, Veggetti et al., 1993). In the larger fish species like muskellunge (*Esox masquinongy*) and rainbow trout (*Salmo gairdneri*) the muscle grew primarily by stratified hyperplastic growth post hatching, whereas hypertrophic growth followed after recruitments of new fibers had ceased (Weatherley et al., 1988). In Atlantic salmon, stratified hyperplasia started prior to hatching (Stickland et al., 1988), and this is earlier than what is observed in smaller pelagic fish. In ballan wrasse stratified hyperplasia was observed from 4 dph (Berg et al., 2012), whereas in cod (Galloway et al., 1999), European sea bass (*Dicentrarchus labrax*) (Veggetti et al., 1990) and gilthead sea bream larvae (*Sparus aurata*)(Rowlerson et al., 1995) it appeared shortly after the onset of exogenous feeding. Stratified hyperplasia is the main process for an increased number of muscle fibers after hatching in fish teleost fish larvae (Johnston, 2006). Alongside the stratified hyperplastic growth in ballan wrasse and cod larvae was the hypertrophic growth (Vo et al., 2016, Berg et al., 2012).

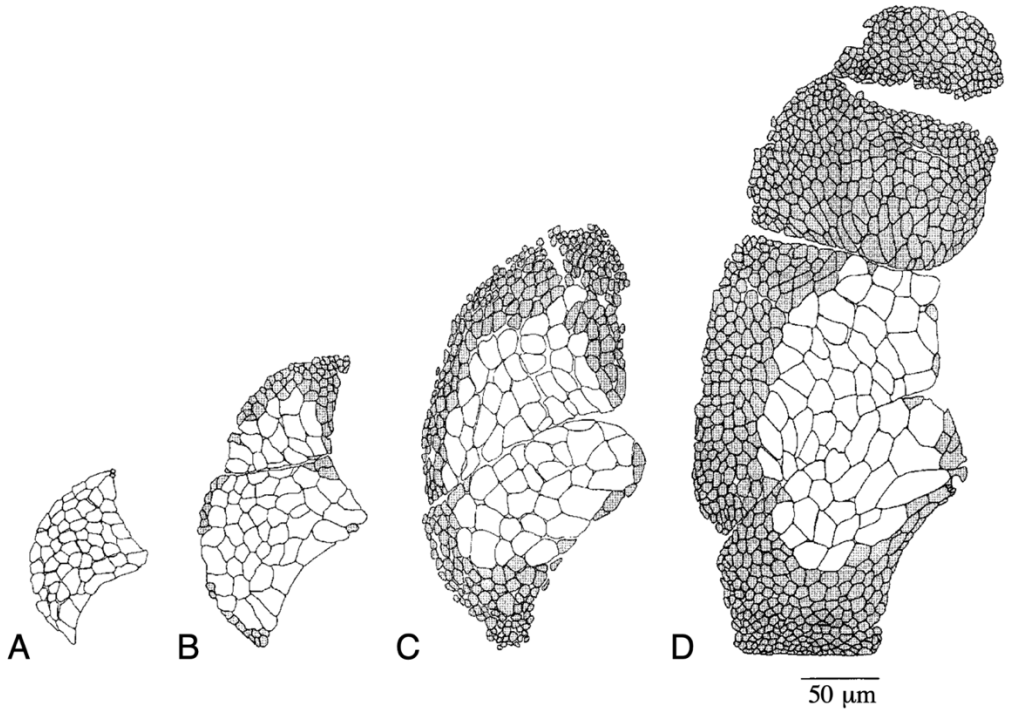


Figure 1.2. Transverse drawing of *camera lucida* muscle development at A) 5 dph (4.4 mm SL), B) 17 dph (5.3 mm SL), C) 31 dph (6.5 mm SL) and D) 31 dph (8.4 mm SL). Shaded fibers belong to small fiber zones. Scale bar 50 µm. Dorsal at the top, lateral to the left and horizontal septum at the bottom. Obtained from (Galloway et al., 1999).

1.2.2 Larval and muscle growth in relation to environmental conditions

Muscle growth is affected by diet composition, temperature, light and feeding regimes (Johnston et al., 2011, Johnston, 1999). Inadequate larval diets and variation in environmental conditions affect somatic growth rates in fish. An increased somatic growth rate is related to an increase in hyperplastic growth in Atlantic cod (Galloway et al., 1999), common carp (*Cyprinus carpio*) (Alami-Durante et al., 1997) and pike perch (Ostaszewska et al., 2008). The growth potential in juvenile and adult fish is thought to be improved by the increased somatic growth rate and hyperplastic growth in the early stages (Galloway et al., 1999, Ostaszewska et al., 2008, Valente et al., 2013). It is therefore essential to know how the muscle grow and how the growth in the fish larvae is affected by environmental conditions and diets in the early stages.

Cod larvae fed copepod nauplii had a significantly higher somatic growth rate corresponding with higher hypertrophic and hyperplastic growth in comparison to rotifer-fed cod larvae (Vo et al., 2016). In ballan wrasse, a higher hyperplastic and hypertrophic growth was obtained in the larvae fed copepods compared to the larvae fed rotifers (Berg et al., 2012). The nutritional compositions and values of the feed hence affects larval growth rates as well as the muscle growth and can thus affect the swimming capacity. The larvae need a well-developed skeletal muscle to be able to catch preys and to avoid predators and how the muscle grow and is affected by diet composition in lumpfish larvae is still not known. It is in the larval phase the different diets with live preys are provided, and the larvae experience a very rapid increase in body mass (Johnston et al., 2011). Since the muscle tissue is the most rapidly growing tissue in fish (Osse and Van den Boogaart, 1995), knowledge about the muscle growth in the larval stage can

therefore be of importance for growth at later stages (Johnston et al., 2011). By investigating the muscle growth mechanisms in the larvae, it can provide information about how the skeletal muscle grows and what feed that might be optimal.

1.3 Aims and hypothesis

The present study was motivated by the fact that farming of lumpfish is still escalating, but knowledge about the larval development and nutritional requirements are still poorly understood. The purpose of this study was to optimize start-feeding regimes of lumpfish and to describe muscle development and growth. The aims for the present experiment were:

Aim 1: Evaluate dietary effect of different start-feeding diets (copepods, *Artemia*, cirripedia and formulated diet) and feeding regimes on growth, survival and growth patterns of skeletal muscle in lumpfish larvae (*C.lumpus*).

Aim 2: Describe the skeletal muscle development (and growth) in *C. lumpus* larvae.

This was studied by conducting a start-feeding experiment with five different feeding regimes (2-35 dph) consisting of four different larval diets (cirripedia, copepods, *Artemia* and formulated diet). In previous experiments with lumpfish, start-feeding with *Artemia* increased larval growth and survival (Marthinsen et al., 2018, Rian et al., 2019, Hanssen et al., 2018). The larval growth is linked to the muscle growth, and is strongly correlated to the body size of fish larvae (Vo et al., 2016). Based on this knowledge the following was hypothesized:

Hypothesis 1: Feeding with *Artemia* will provide the highest larval growth, survival and muscle growth in both hyperplasia and hypertrophy mechanism.

Hypothesis 2: Muscle growth dynamics (hyperplasia and hypertrophy) have a stronger relation to larval body size rather than larval age.

Larval growth was evaluated by dry weight (DW), standard length (SL) and daily weight increase (DWI). The muscle growth was examined through morphology, counting muscle cells, and measuring their cell size. This was further evaluated in relation to diet and SL.

This start-feeding experiment was part of the project "Optimalisert startfôring av rensefisk (STARTRENS)" (https://www.fhf.no/prosjekter/prosjektbasen/901561/). The project was a collaboration between SINTEF Ocean AS and NTNU and was mainly funded by FHF (Fiskeri- og havbruksnæringens forskningsfinansiering). The "STARTRENS" aim was to optimize the start feeding regimes in cleaner fish (*Cyclopterus lumpus* and *Labrus Bergylta*). There were two other master students involved in the lumpfish experiment: Sunniva Brevik Kværnø (nutritional condition) and Saba Akbar (lipid analysis).

2 Materials and methods

2.1 Start feeding of lumpfish larvae

2.1.1 Lumpfish larval rearing and experimental setup

The start-feeding experiment and analysis took place in the laboratories at Sealab NTNU and SINTEF Fisheries and Aquaculture in Trondheim from September 9 to October 14, 2020. Unfertilized lumpfish eggs from 7 females were supplied from Akvaplan-niva in Tromsø. After arrival at the rearing lab at NTNU (Sealab) in Trondheim, eggs were pooled together in a bucket and milt from 2 males were used for fertilization. Fertilized eggs were incubated in 15 egg incubators containing 300 mL seawater (Sterner) at 10 °C (Figure 2.1). As eggs hatched, the larvae were transferred from the egg incubator to their assigned tank (Figure 2.1) through a tube.

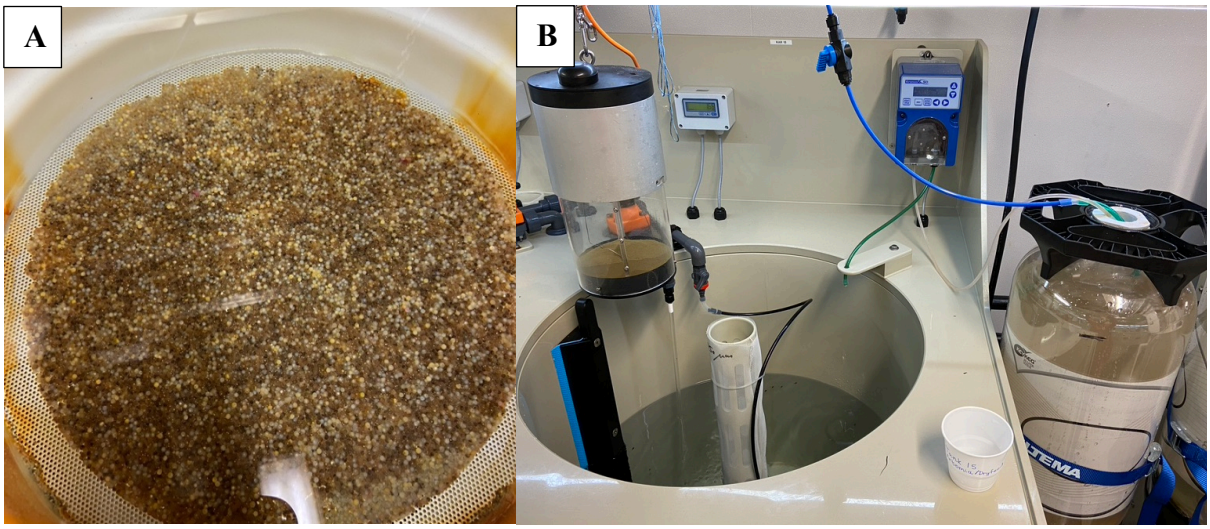


Figure 2.1 A) Lumpfish eggs in the egg incubator and B) experimental set up of the tank with a sieve, cleaning arm, feeding automat and live prey reservoir. Photos by Marte Lindskog.

Cylindrical tanks with flat bottoms contained 100 L of seawater (33-34 ppt salinity) and an estimated density of 100 larvae/L. Seawater was pumped from 77 meters depth, 800 meters out in the Trondheimsfjorden and filtered through a sand filter and a membrane filter of 1 µm. The water in each rearing tank was aerated by tubes close to the outlet in the bottom of the tank. The water exchange rates were based on the feed type (Appendix 1: Table A1) and were established based on experience from an earlier experiment (Marthinsen et al., 2018). Oxygen saturation and temperature were measured every day near the bottom (YSI ProODO, USA). Each tank was illuminated with constantly dimmed lightning with LED (30% of max intensity) during the whole

experimental period (24/24 hours). From 2 dph until 22 dph silicon plates were placed hanging down for the lumpfish to attach to.

An aerated feeding reservoir (KeyKeg, 20 L) with live prey was stored next to each tank and the live preys were transferred constantly from their reservoir to the tank through a peristaltic pump which was part of the tank setup. A feeding automat (Sterner Fish Tech AS, Norway) for formulated diet was installed over each tank and they were programmed over the central operating facility (Normatic AS) at Sealab NTNU. The automatic pump distributed the formulated diet into the tanks.

Excess feed and dead fish from the tank bottom and walls were removed once per day and thereafter two times per day as the feed density increased. Additionally, from 8 dph a cleaning arm was cleaning the tanks one time each day at the slowest speed (one tank round per hour). After 2 weeks the speed was set to 2 tank rounds per hour. The dead fish were counted and registered every day. In the middle of the tank covering the outlet was a sieve with different mesh size based on the feed type. The mesh size changed throughout the experiment according to the change in feed type, to let live prey be flushed out over time (Appendix 1: Table A1).

2.1.2 Feeding regimes

Three live preys (*Artemia*, cirrpedia and copepods) and one formulated diet was used to create five feeding regimes (Table 2.1). Five feeding regimes were used in this start-feeding experiment of lumpfish (2-34 dph) (Table 2.2). The first group of larvae received *Artemia* nauplii (Instar III meta-nauplii), the second cirripedia nauplii, the third copepod *Acartia tonsa* (stage n5/n6) and the fourth formulated diet (Gemma micro). The last larval group received copepod nauplii at first, before a change to cirripedia nauplii from 10 dph. These groups will from now be referred to respectively as *Artemia* or Art group, cirripedia or cir group, copepod or cop group, FD group and cop/cir group. All five feeding regimes weaned over to formulated diet (Gemma micro 300, Skretting) from 21 dph and until the experiment was terminated at 35 dph. Three replicate feeding tanks were used for each feeding regime, providing a total of 15 tanks. The feeding regime is presented in Table 2.2.

Table 2.1 Live preys and formulated pellets used in the lumpfish start-feeding experiment. A) *Artemia* nauplii (photo by Juan Camilo Jaramillo) (Hamidi et al., 2014), B) cirripedia nauplii (photo by Marte Lindskog), C) copepods *A.tonsa* (photo by Tora Bardal) and D) Gemma micro (photo by Skretting AS).

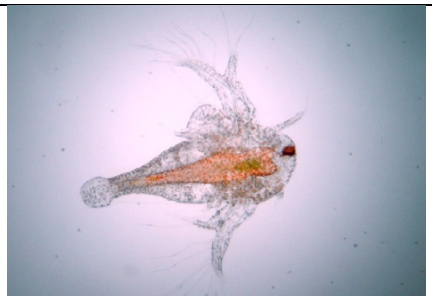
Feed type		**Size**
A) *Artemia* nauplii		800 µm after 24 hours enrichment (Baert et al., 1996)
B) Cirripedia nauplii		350 µm long and 150 µm wide after thawing and revitalization for 6 hours. (Planktonic AS, Norway)
C) Copepod *A.tonsa*		185-394 µm in stage n5/n6 (C-feed AS, Norway).
D) Formulated diet (Gemma micro)		150 µm and 300 µm (Skretting AS, Norway)

Table 2.2. Feeding regimes for start feeding of lumpfish (*C.lumpus*) from 2 to 35 dph. Five different feeding regimes were used, and each feeding regime had three replicate tanks (*n*=3). Sampling times for histology and growth is marked with an X. The overlapping in each feeding regime indicates weaning periods.

Date	**September 2020**																					**October 2020**														
	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Dph	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Sampling			X							X						X						X								X					X	X
***Artemia* (*n*=3)**			*Artemia*																																	
																						Gemma micro 300														
Cirripedia (*n*=3)			Cirripedia																																	
																						Gemma micro 300														
Copepod (*n*=3)			Copepods (*Acartia tonsa*)																																	
										Gemma micro mix												Gemma micro 300														
Formulated diet (*n*=3)			Gemma micro 150																																	
																						Gemma micro 300														
Copepod/ Cirripedia (*n*=3)			Copepods (*Acartia tonsa*)																																	
																						Cirripedia														
																						Gemma micro 300														

2.2 Live feed production and diets

2.2.1 *Artemia*

Every morning *Artemia* cysts (EG ® INVE Aquaculture, Thailand) were weighed using a scale (Mettler Toledo GmbH D-72458 Albstadt, Germany) and put into a 60 L cylindroconical tank with seawater (25-29°C). The seawater was heated using an electronic aquarium heater (EHEIM thermocontrol e300, Germany), and the tanks were placed under fluorescent tube light. According to the producers' recommendations and our own preliminary results a hatching efficiency of 260 000 nauplii g^{-1} dry weight of cysts was used to calculate the amount of cysts to use. The cysts were kept in heavy aeration for 24 hours, before newly hatched *Artemia* nauplii were separated (SEP-Art Magnetic Artemia SEPARATOR, Australia) from the unhatched cysts and put into a new cylindroconical tank for enrichment. *Artemia* nauplii were enriched according to the producer's recommendations, with 10 g Larviva Multigain (Biomar AS, France) twice in 24 hours. A plankton net (60μm) was used for harvesting. The *Artemia* nauplii were transferred to the feeding reservoirs two times per day and the amount in each tank is presented in Appendix 2, Table A2. A protocol for the *Artemia* is presented in Appendix 3.

2.2.2 Cirripedia

Frozen pellets of cirripedia nauplii and cryoprotectant agent (CPA) called Cryoplankton (Planktonic AS, Norway) were stored in liquid nitrogen (-196 °C). Cryoplankton were thawed in seawater and CPA removed by rinsing with seawater for approximately 3 minutes before a plankton net (100 μm) was used to capture the cirripedia nauplii's (Appendix 4). A 55 L cylindroconical tank (aerated) with pre-cooled seawater (5°C) was used to store the nauplii's until larval feeding. Minimum six hours was needed for revitalization. Nauplii's were transferred into the feeding reservoirs two times per day and the amount in each tank is presented in Appendix 2, Table A2. There were 50 million nauplii per kilo Cryoplankton.

2.2.3 Copepods

Cultures of *Acartia tonsa* nauplii (C-feed AS, Norway) was stored in a 1 m^3 plastic container in a temperature regulated room (10 °C). New deliveries were received twice a week throughout the experiment, along with the microalgae *Rhodomonas baltica* (C-feed AS, Norway). Both *A.tonsa* and *R.baltica* were kept in heavy aeration and *R. baltica* was used to feed the *A.tonsa*. *A.tonsa* nauplii was harvested using a sieve (53 μm) into three buckets which together contained enough *A.tonsa* for one day of feeding (Appendix 5). The amount of nauplii's put into each tank is presented in Appendix 2, Table A2.

2.2.4 Formulated diet

The formulated diet was GEMMA Micro 150 and 300 μm (Skretting AS, Norway). GEMMA micro is a formulated diet specialized to provide optimal nutrition for marine fish larvae from early dry feed weaning (Skretting AS, Norway). The amount of feed was predetermined (Appendix 2: Table A2) and placed in feeding automats (Sterner Fish Tech

AS, Norway) above the tanks. An automatic pump distributed the formulated diet in intervals throughout the day. The same formulated diet (Gemma micro 300) was used towards the end of the experiment (21 to 35 dph) for the other feeding regimes as well (see Table 2.2).

2.3 Larval sampling

Right after sampling, all the sampled larvae were sedated in tricaine methane sulfonate (MS-222 Finquel®, Agent Chemical Laboratories Inc., USA). Larvae sampled for histology analysis were pooled and fixated in 4 % paraformaldehyde (PFA) in phosphate buffered saline (pH 7.4, Apotekproduksjon AS; Norway) and then placed at 4 °C and stored until further analysis. They were therefore fixated before standard length measurements were taken. Larvae sampled for larval growth (dry weight (DW) and standard length (SL)) was rinsed in distilled water to remove salt particles and stored until further analysis. The SL of these larvae were taken right after the sampling and rinsing. The sampling days represent when weaning to a new feed starts, or a diet is finished (Table 2.2). On the last day of the experiment (35 dph), after the tanks were emptied and the larvae euthanized with tricaine methane sulfonate (MS-222 Finquel®, Agent Chemical Laboratories Inc., USA), 250 larvae were sampled from each tank to check for bias. All the remaining larvae were also counted. Number of larvae sampled and analyzed are given in Table 2.3.

Table 2.3 Number of sampled and analyzed larvae for growth and histology. On 2 dph the number represents the total sample size as the treatments had not been distinguished yet. From 9 to 25 dph the sample size is from each tank for growth and from each treatment for histology.

Dph	**2**	**9**	**15**	**21**	**29**	**34**	**35**
Growth							
Sampled	15	5	5	10	15	15	250
Analyzed	15	5	5	10	15	15	250
Histology							
Sampled	15	15	15	15	15	0	15
Analyzed	5	5	0	5	0	0	5

2.4 Larval growth and survival

2.4.1 Growth

Larval growth was evaluated based on dry weight (DW) and standard length (SL) from seven different sampling days (Table 2.3). SL was measured from the tip of the snout and back to the end of the notochord by the software ImageJ. The images processed in ImageJ were taken in a stereo microscope (Leica MZ75, Leica Microsystems, Germany; Zeiuss Axiocam ERc 5c, Zeiss Inc., Germany) with 0.63x magnification. Images were furthermore used to assess external morphology. For the same larvae, dry weight was measured in pre-weighed tin capsules with an ultra-microbalance weight (UMX2 Ultra-microbalance, Mettler-Toledo, USA), after the larvae had been dried for 2 days (48h) at

60°C. Specific growth rates (SGR) were calculated on specific sampling intervals with the following equation (Houde, 1981):

$$SGR = \frac{lnW_2 - lnW_1}{t_2 - t_1}$$

Where t_2 and t_1 are the time (dph) and W_2 and W_1 are the average larval dry weight for each tank. Based on the calculated SGR-values, percentage daily weight increase (DWI) was further calculated (Houde, 1981):

$$DWI = (e^{SGR} - 1) * 100\%$$

2.4.2 Survival

The total number of larvae in each tank and treatment throughout the experiment was calculated. Based on the number of larvae left in the tanks, the daily registrations of dead larvae and the number of larvae sampled, the survival (S_t) in each tank was calculated:

$$S_t = \frac{N_t}{N_0} * 100\%$$

where N_0 is the total number of larvae at the beginning of the experiment and N_t is the total number of larvae alive at a given time (dph) in the experiment.

2.5 Muscle analysis

Histological analyzes of the muscle development were analyzed on transverse sections, cut 500 µm behind the anus in lumpfish larvae at 2, 9, 21 and 35 dph (Table 2.3). Fixated samples were rinsed in phosphate-buffered saline (PBS) and images were taken with 0.63x magnification on a stereo microscope (Leica MZ75, Leica Microsystems, Germany; Zeiuss Axiocam ERc 5c, Zeiss Inc., Germany) for SL measurements. The larvae were further cut in two in front of the anus, and the tail-part was dehydrated and embedded in plastic (Technovit® 7100, Kulzer, Germany; Appendix 6). 2 µm transverse sections were made with glass knives by a microtome (Reichert Ultracut S, Leica Microsystems, Austria). The sections were further stained with 0.05 % toluidine blue (Honeywell Riedel-de-Haën™, Germany) for 30 seconds and dried before a glass slide cover was pasted on the slide. The slides were scanned with a digital scanner (NanoZoomer SQ, Hamamatsu Photonics, Japan) in three 0.5 µm layers at 40x magnification. The reference point for the transverse section was behind the anus 500 µm (Figure 2.2). The upper left quadrant of the transverse section was used for analyzing the muscle growth. In the quadrant, the red and white fibers were distinguished (Figure 2.3).

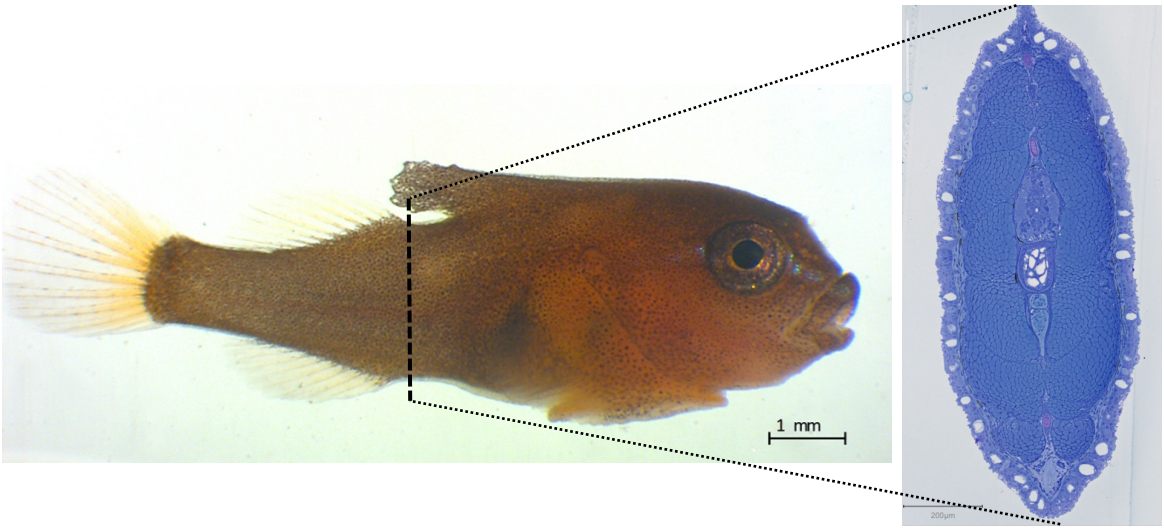


Figure 2.2. Overview of the reference point where the transverse section were made in *C.lumpus*. The transverse section is from a larva fed *Artemia* on 9 dph.

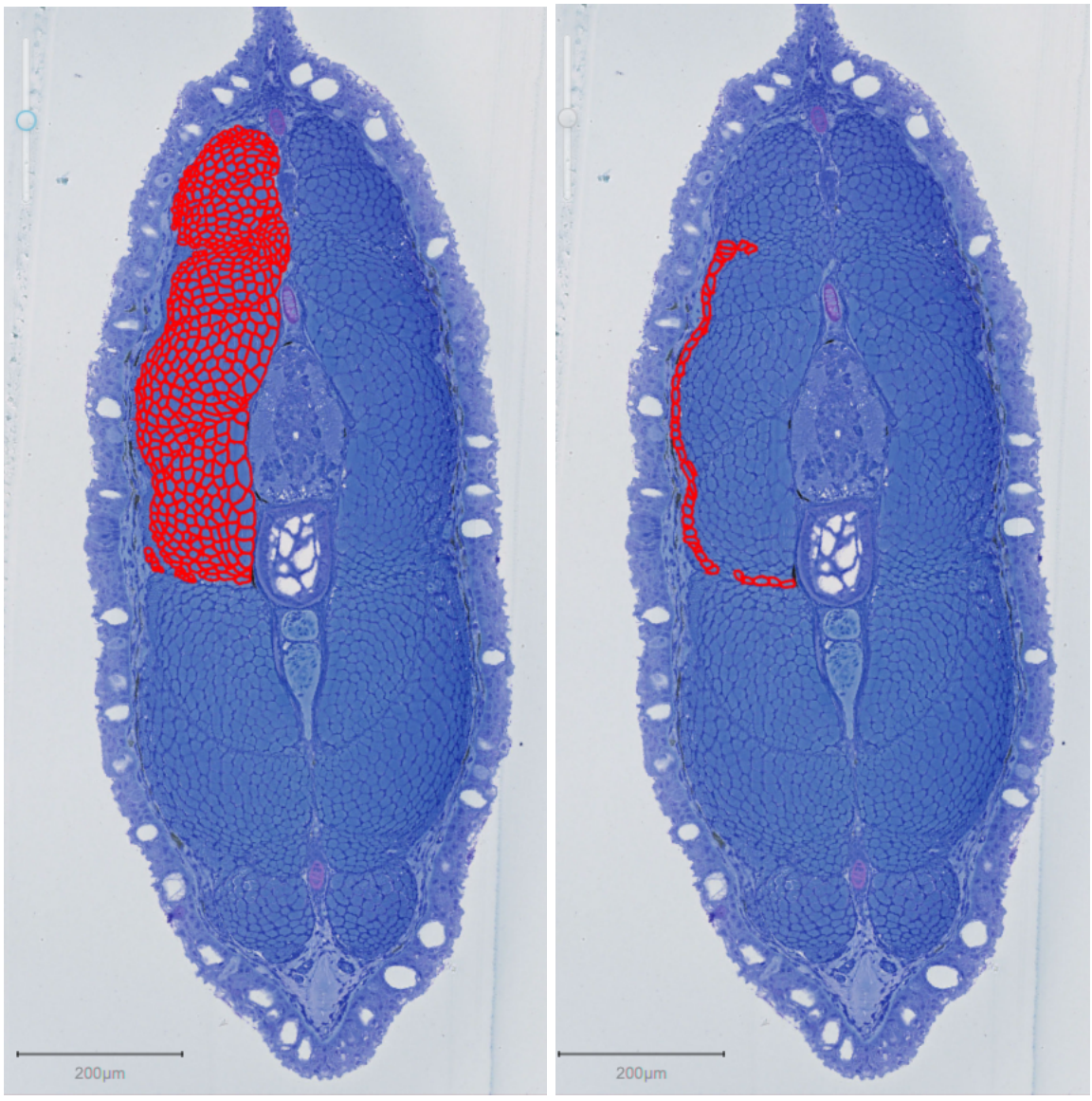


Figure 2.3. Transverse section from *C.lumpus* larva fed *Artemia* on 9 dph. The upper left quadrant was used for analysis. White (left) and red fibers (right) were measured by using QuPath-0.2.3.

Red and white muscle fibers were counted and the individual fiber size in the upper left quadrant (Figure 2.3) were measured as described by Vo et al. (2016) using the software QuPath-0.2.3. Parameters measured and calculated by Quapath-0.2.3 were fiber size and the number of fibers. The fiber size was used to estimate if the fibers grew in size (hypertrophy) and the 350 largest white fibers, and 30 largest red fibers were used because that was the lowest number of fibers counted in one larva. The total area of both red and white muscle was measured by adding all the fibers area together. The number of red and white fibers were used to evaluate if the muscle recruited new fibers after hatching (hyperplasia). Mosaic hyperplasia was defined as small white fibers (<100 µm) formed in between already existing white fibers. The total muscle area, the number of fibers and their size were further tested statistically to find if there was a correlation to larval size. The diet effect on muscle growth was evaluated by comparing the number of fibers and the mean fiber size of 350 largest white and 30 largest red fibers between larvae of the same size fed different feed.

2.6 Statistical analysis

Statistical analyzes and graphs were performed and made in Sigmaplot 14.0. A significant level of $\alpha = 0.05$ was used for all statistical tests, except for the correlations where $\alpha = 0.01$ was used. For the percentage survival data an arcsine transformation was performed before applying the statistical test. Tables were made in Microsoft Office Excel 2021 and all mean values are presented with standard errors ($\pm$ SE).

To assess if a sample was likely to originate from a normal distribution the Shapiro-Wilk test was applied. Homogeneity of variance was further tested with the Brown-Forsythe test to estimate the similarity between population variances. In homogenous normally distributed data, the differences in the group means were compared by applying a one-way analysis of variance (ANOVA) followed by a Tukey test to pairwise compare the means of all the groups. The non-parametrical Kruskal-Wallis test followed by Dunn's test were applied if not.

Analysis of covariance (ANCOVA) was applied to investigate if the muscle development and mosaic hyperplasia pattern were significantly affected by the treatment and/or SL. A log transformation was used on the general muscle development data, and r-square transformation was used for the mosaic hyperplasia pattern. The transformations were applied because normality and/or equal variance failed.

3 Results

3.1 Larval growth and survival

3.1.1 Dry weight

Mean larval dry weight was 0.91 ± 0.05 mg on 2 dph before the different feeds were introduced (Figure 3.1; Appendix 7, Table A3). The lumpfish larvae fed *Artemia* had the highest growth rate from start and continued to grow fast until the weaning at 21 dph. At 15 and 21 dph the *Artemia*-fed larvae had a significantly higher DW compared to the larvae from the other feeding regimes ($p<0.05$). At 21 dph and 29 dph the *Artemia*-fed larvae had a slower growth rate, but it increased again at 35 dph. The larvae fed cirripedia had the lowest DW at 9 dph, significantly lower than the *Artemia*-fed larvae ($p=0.016$). At 15 dph the cirripedia-fed larvae had a significantly higher DW compared to the FD-fed larvae ($p=0.031$) but the highest growth in the cirripedia-fed larvae were at 21 dph and until the experiment ended (35 dph). The DW in the larvae from the cop/FD group was generally low at 9 and 15 dph, and at 21 dph the larvae had a significantly lower DW compared to the larvae from the other feeding regimes ($p<0.05$). At 29 and 35 dph the DW had increased approximately by a 2-and 3-fold respectively. The larvae from the cop/cir group had a slow growth at 9 and 15 dph when fed copepods, but the growth increased after the change to cirripedia, which was fed exclusively from 17 dph. The DW continued to increase at 21, 29 and 35 dph. The larvae fed exclusively with FD obtained a slow growth in DW at 9 and 15 dph, but after changing exclusively to a bigger FD (15 dph) the growth increased at 21, 29 and 35 dph. The FD-fed larvae had a similar DW at 35 dph as the larvae from the cop/FD group and the cop/cir group. The highest mean DW at the end of the experiment (35 dph) was in the *Artemia*-fed and cirripedia-fed larvae. The larvae from those two treatment groups were similar in DW ($p>0.05$) but significantly larger compared to the larvae from the three remaining treatment groups ($p<0.05$).

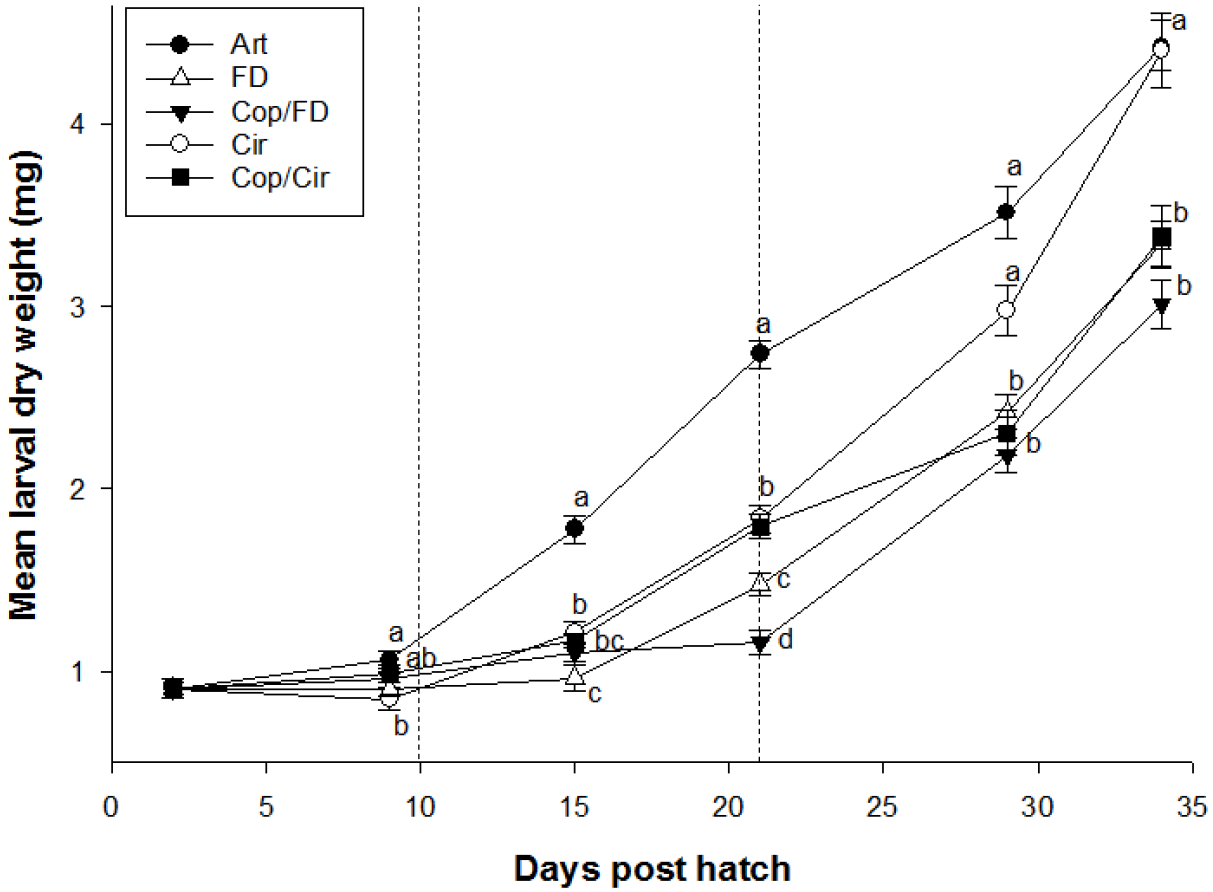


Figure 3.1 Mean larval dry weight (mg) of *C.lumpus* larvae. For 2 dph *n*=15 larvae, and for 9 and 15 dph (*n*=15), 21 dph (*n*=30), and for 29 and 34 dph (*n*=45) per treatment. The dashed lines denote when weaning to formulated diet starts. At 10 dph, weaning started for the larvae in the cop/FD, FD and cop/cir group and at 21 dph larvae from Art and Cir started weaning. Error bars are ± standard error (SE) and significant differences between treatment groups ($p < 0.05$) are denoted by different letters.

3.1.2 Daily weight increase

From 2 to 9 dph the larvae in the *Artemia,* cop/FD and cop/cir group increased in larval DWI, whereas the larvae in the cirripedia and FD group had a negative trend (Figure 3.2; Appendix 8, Table A4). No significant differences were observed between the larvae in the different treatments in the 2-9 dph interval. The *Artemia*-fed larvae had the highest DWI in the start and increased further and was highest in the time interval 9-21 dph. Significantly higher compared to the larvae from the cop/FD and FD group ($p<0.05$). Furthermore, the *Artemia*-fed larvae had the lowest DWI at the 21-34 dph time interval, significantly lower than in the larvae from the cop/FD and cirripedia group ($p<0.05$). The larvae fed cirripedia had a negative DWI trend between 2 and 9 dph but the DWI increased after this period and throughout the experimental period. The DWI in the cirripedia-fed larvae were similar in the intervals 9-21 dph and 21-34 dph. The cop/FD-fed larvae obtained a slow DWI growth at the 2-9 dph and 9-21 dph time intervals, with a significantly lower DWI compared to the larvae fed *Artemia* and cirripedia in the 9-21 dph time interval. In the following dph interval (21-34 dph), the DWI in the cop/FD larvae had increased considerably and was significantly higher compared to the *Artemia*-fed larvae ($p=0.007$). The cop/cir larvae had a similar DWI between the two dph

intervals 9-21 and 21-34 dph whereas the FD- fed larvae had a higher increase in DWI in the 21-34 dph interval. For the whole experimental period, the DWI (5%) of the larvae fed *Artemia* and cirripedia was significantly higher compared to the DWI in the larvae from the other treatment groups with 4% DWI (p<0.05).

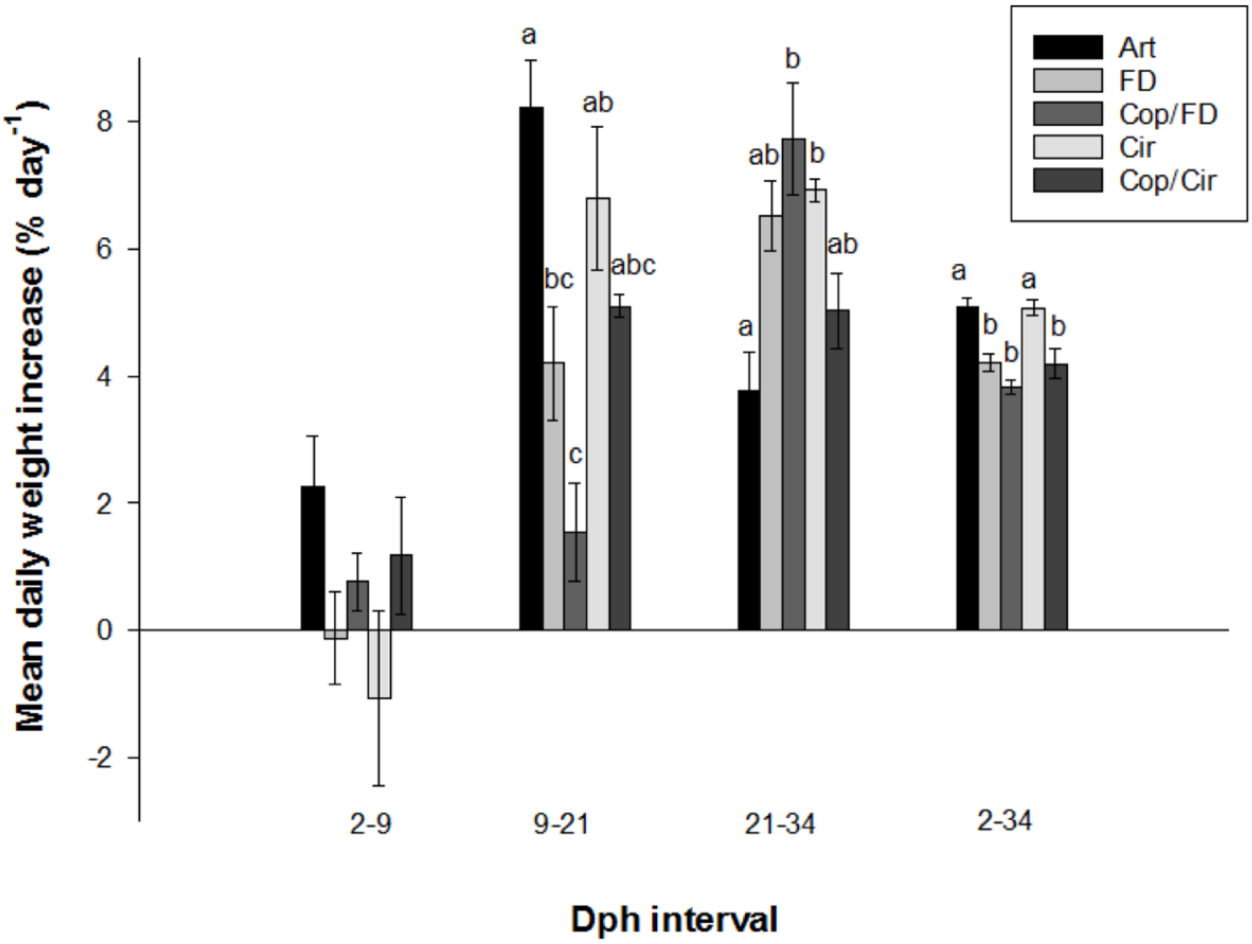


Figure 3.2 Mean daily weight increase (%/day^{-1}) in *C.lumpus* from 2-34 dph. For 2 and 9 dph (n=15), 21 dph (n=30) and 34 dph (n=45). The time interval to the right represents the whole experiment period (2-34 dph). Error bars are ± standard error (SE) and significant differences (p< 0.05) between treatments on the same time interval are shown with different letters.

3.1.3 Standard length

Mean standard length of 2 dph aged larvae was 5.94 ± 0.10 mm (Figure 3.3; Appendix 9, Table A5). All larvae had similar SL on 9 dph (p=0.617) but on 15 dph and throughout the experimental period the *Artemia*-fed larvae had the highest SL. The SL was significantly higher compared to all the other larvae on 15 (except the cop/cir larvae) 21, 29, 34 (except the cirripedia larvae) and 35 dph. The larvae fed exclusively with FD had the shortest SL at 15 dph. At 21 dph the FD-group passed the cop/FD larvae in SL as the larvae in the cop/FD group had a stagnated growth between 15 and 21 dph. At 21 dph, the SL of the larvae in the cop/FD group was significantly lower compared to the larvae in the other treatment groups except the ones fed FD (p=0.051). The larvae in the cop/cir group obtained almost no growth between 21 and 29 dph, but at 34 dph the larvae's SL had increased. At 29 and 34 dph the larvae in the *Artemia* and cirripedia group had a significantly higher SL compared to the larvae in the remaining groups

(p<0.050) which were similar in SL (p>0.050). The larvae in the *Artemia*- and cirripedia group gained the highest SL but in the larger sample size at 35 dph (Figure 3.3; Appendix 10; Table A6) the *Artemia*-fed larvae had a significantly higher SL compared to the larvae in the other treatment groups, including the cirripedia-fed larvae. The cop/cir and FD-fed larvae had a similar SL (p=1.000), and the SL was significantly lower than the *Artemia* and cirripedia larvae, but significantly higher compared to the cop/FD larvae with the shortest SL. The SL of the cop/FD-fed larvae was significantly lower compared to all the larvae in the other treatment groups (p<0.050).

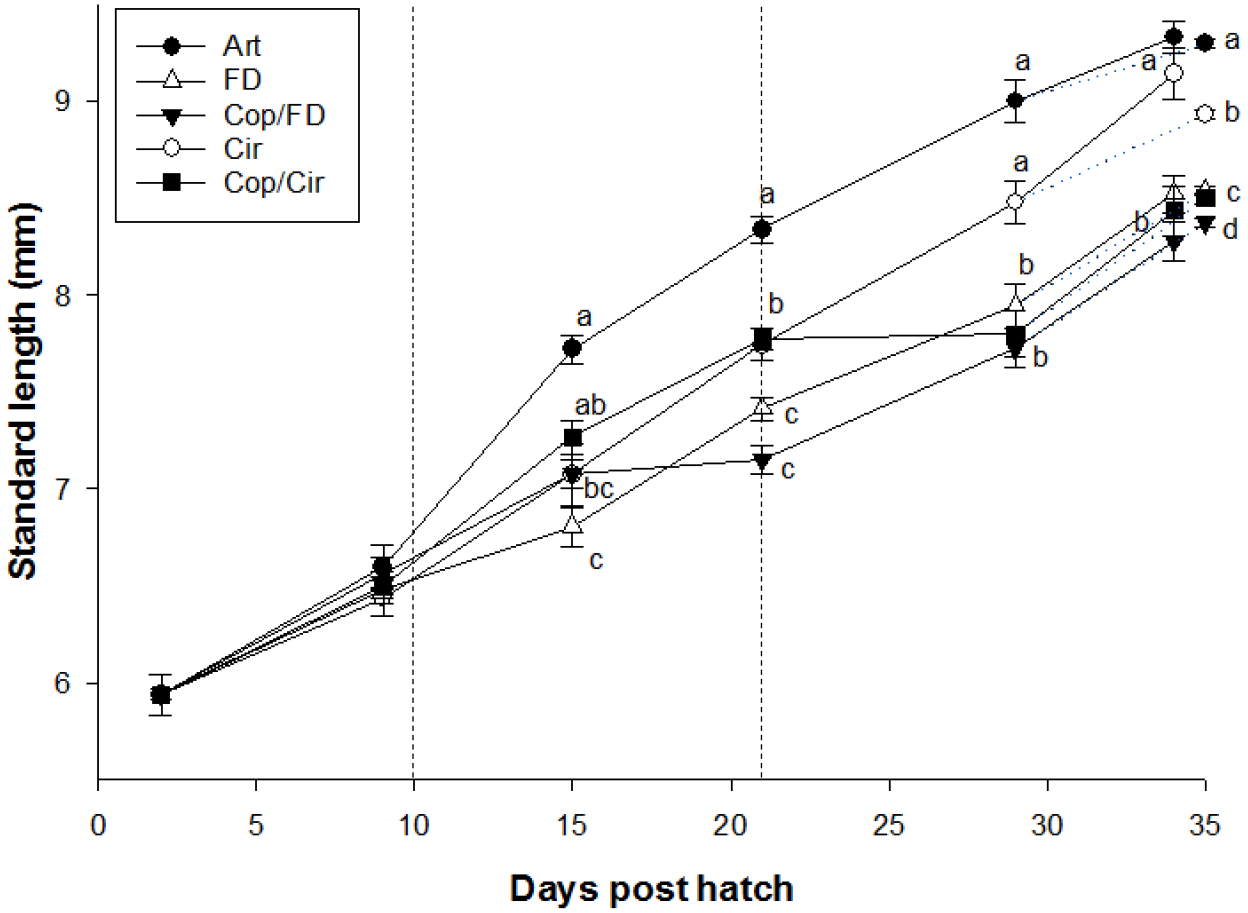


Figure 3.3 Mean standard length (mm) of *C.lumpus* larvae. At 2 dph n=15 larvae, 9 and 15 dph (n=15), 21 dph (n=30), and for 29 and 34 dph (n=45) and 35 dph (n=750) per treatment. The dashed lines denote when weaning to formulated diet starts. At 10 dph weaning started for the larvae in the cop/FD, FD and cop/cir group. At 21 dph Art and Cir started weaning. Error bars are ± standard error (SE). Significant differences (p< 0.05) between treatments are denoted by different letters.

It was a strong positive correlation between SL and DW in the larvae, and the variables increased together (r^2=0.94, P<0.0001). A slower growth was attained in the start when the larvae were small. From 5 to 7 mm SL the DW was approximately 1 mg. From approximately 7 mm SL the lumpfish larvae started to grow in both SL and DW (Figure 3.4).

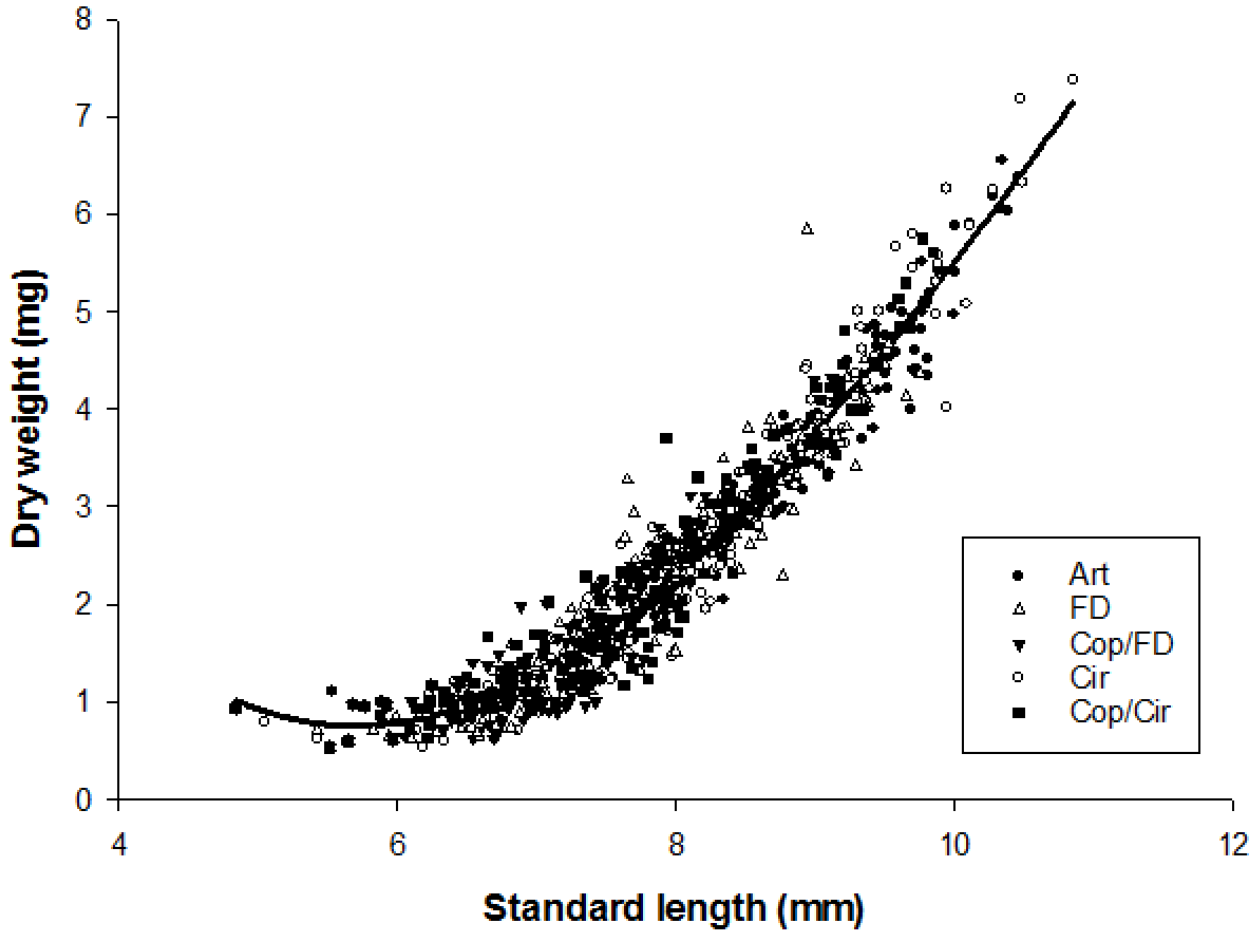


Figure 3.4 Correlation between standard length and dry weight for *C.lumpus*. *n*=166 for *Artemia* and cirripedia and *n*=165 for copepod, cop/cir and FD-group. A global curve fit was added, where polynomial cubic fit was the best (r^2=0.94, *P*<0.0001).

3.1.4 Survival

The mean survival was generally high in all the larvae in the different groups throughout the experiment (Figure 3.5; Appendix 11; Table A7). The larvae in all the groups had a survival over 85%. The cop/FD-fed larvae were the only group under 90% survival at 35 dph and that was significantly lower than the larvae in the *Artemia* group (p<0.05). The larvae from the cirripedia and cop/FD group had a higher mortality from the start of the experiment and the cop/FD larvae continuously decreased in survival throughout the experiment. There had been observed differences in mortality between the tanks from the same feeding regime, thereby the high error bars. At the end of the experiment the larvae in the *Artemia* group obtained the highest survival of 95% and the cop/FD larvae the lowest survival of approximately 85%.

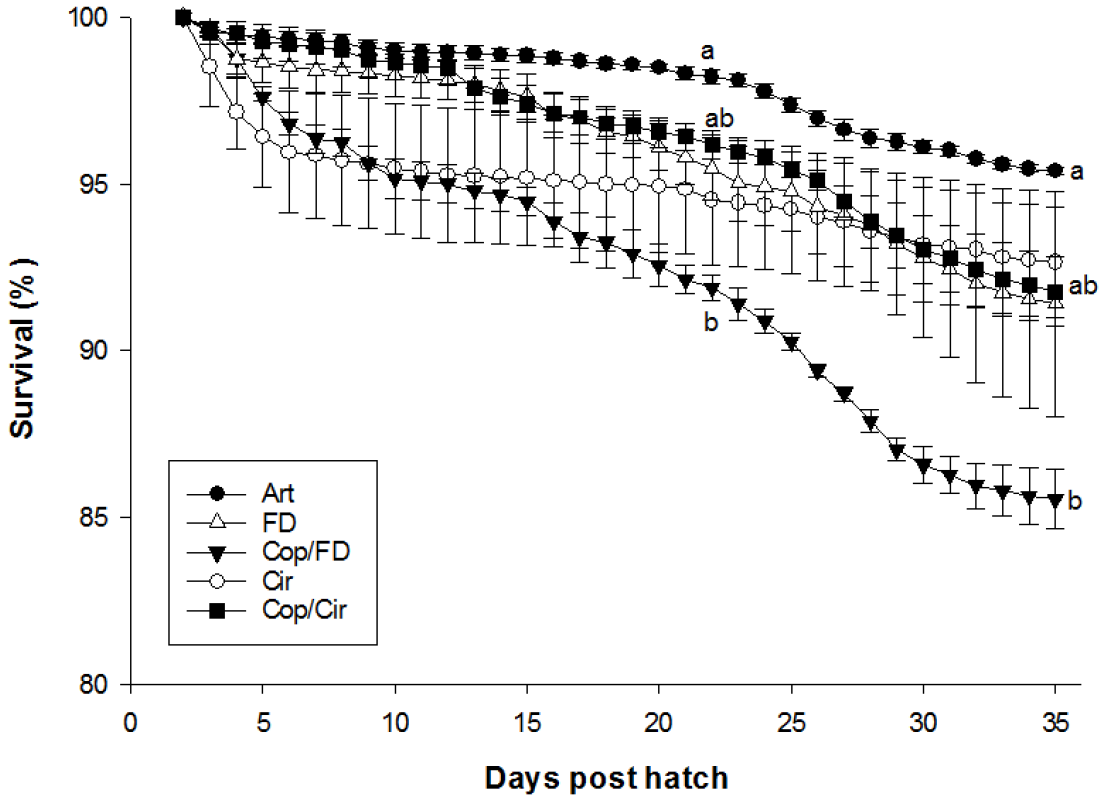


Figure 3.5 Mean survival (%) for each treatment (n=3) with corresponding error bars ± SE. Significant differences between treatment groups are presented with different letters (p<0.05).

3.2 Larval and muscle morphology

Larvae fed *Artemia* obtained the best growth (Section 3.1) and were therefore used to describe the larval morphology (Figure 3.6). At 2 dph (Figure 3.6A) the head and trunk (between head and anus) regions were larger compared to the rest of the body. The tail region was transparent, whereas the head and trunk were yellow and brown with black pigmented cells. The tail region was surrounded by continuous fin fold and the fin rays were visible in the caudal and pectoral fins. Posterior to the eyes was a lighter pigmented stripe and on the ventral side of the larval trunk the suction disk was functional and differentiated. The heart was located on the ventral side of the eye stripe, and posterior to the yolk-sac. At 9 dph (Figure 3.6B) the dorsal fin was more distinguishable from the trunk and had become more overgrown with external tissue. The fin rays were more outlined, and the notochord end was flexing upwards. Posterior to the eye, the stripe had become more visible and shinier and was now visible anterior to the eyes also. The pigmentation of the tail region increased for several larvae but was uneven for many. At 21 dph (Figure 3.6C) the whole larva was fully pigmented. Most of the larvae fed *Artemia* had a red pigmented color, and the larvae fed FD were mostly green. The remaining larvae from the other treatment groups were mainly brown in color. The first dorsal fin was now covered with external tissue and had no visible fin rays. The fin rays in the anal, caudal and second dorsal fin were clearly developed and the fin fold was reabsorbed. The eye stripe had become narrower, but shinier. The size of the larva was clearly bigger, and the tail region was more muscular. At 35 dph (Figure 3.6D) the morphology was similar to 21 dph. The anal, caudal and second dorsal fins had attained a weak and faint pigmentation, and this was observed in several larvae. Pigmentation on the whole larval body was variable according to feeding treatment, as mentioned in 21 dph.

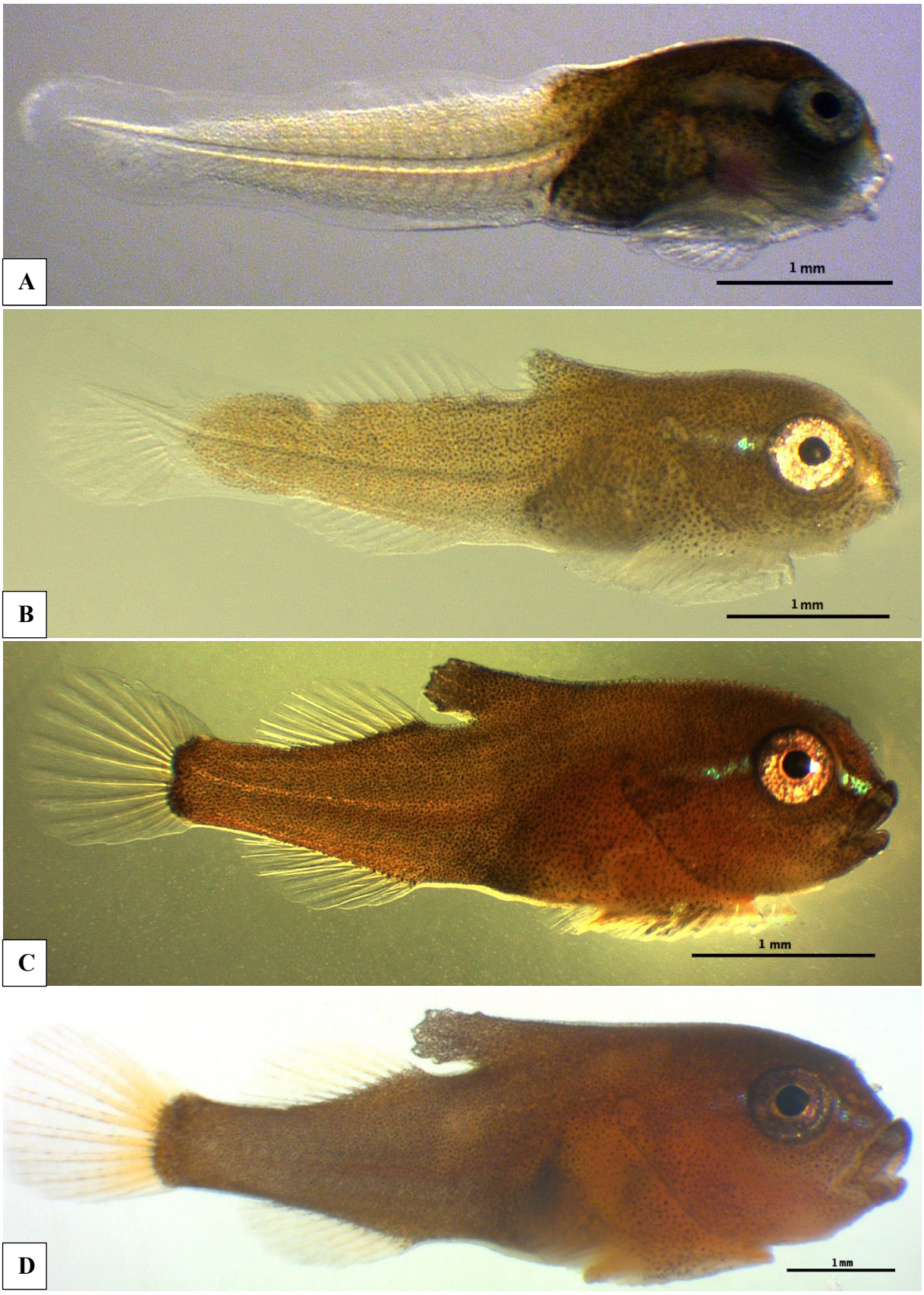


Figure 3.6 External morphology of *C.lumpus* fed *Artemia* from 2-33 dph. A) 2 dph is from before feeding started, whereas B) 9 dph, C) 21 dph and D) 33 dph. Scale bar 1 mm.

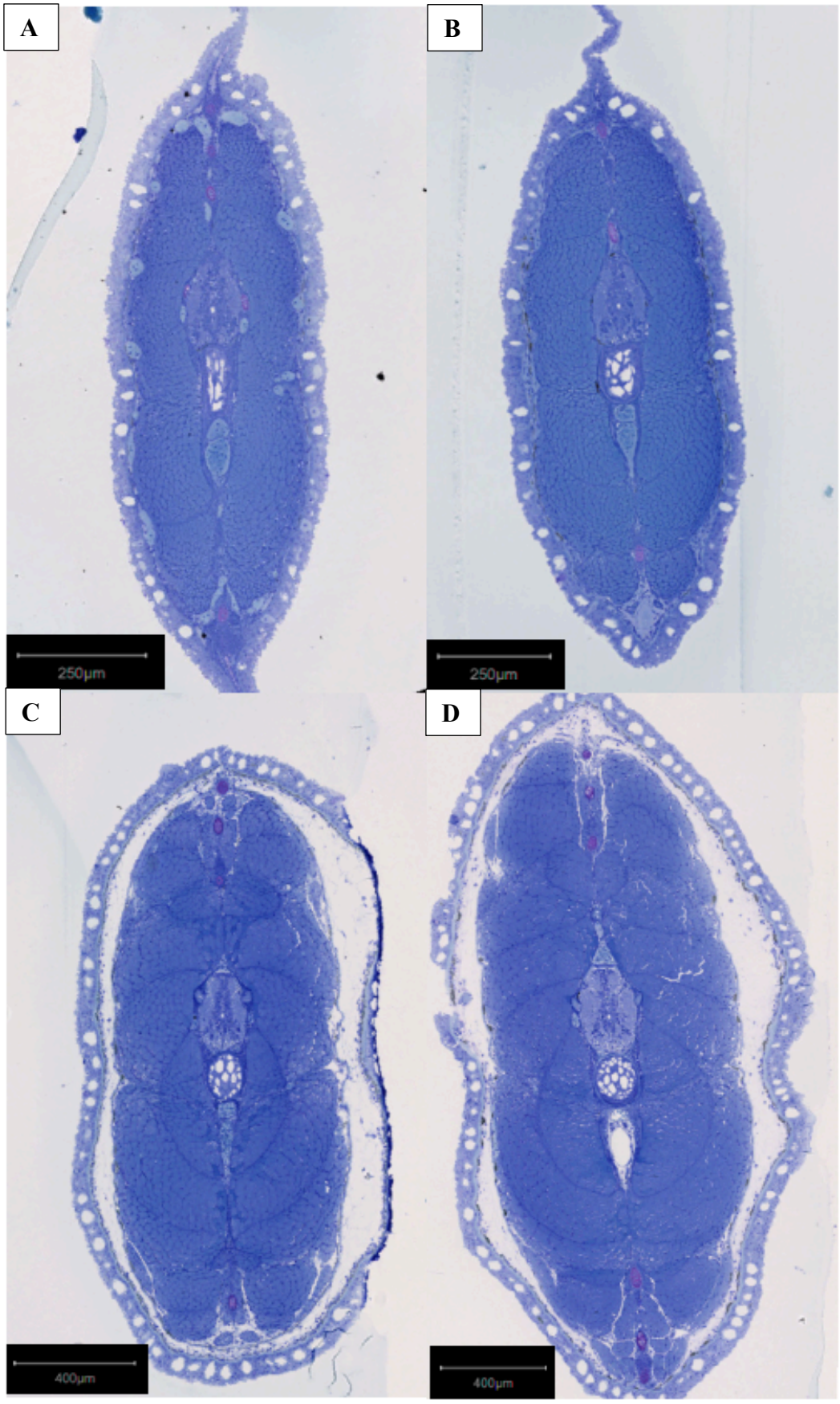


Figure 3.7 Transverse sections of skeletal muscle behind anus in *C.lumpus* larvae. Corresponding to the larvae in Figure 3.6. Sections are from the *Artemia* group as they obtained best growth. A) 2 dph, B) 9 dph, C) 21 dph and D) 35 dph. Scale bar 250 µm in A and B; 400 µm in C and D.

The epaxial quadrant of the myotome grew bigger throughout the experimental period and was wider at 21 and 35 dph compared to 2 and 9 dph (Figure 3.7). At 21 and 35 dph there was a loose connection between the muscle fibers and the skin (Figure 3.7) in almost all the larvae from the different treatments. The connection was tighter in 2 and 9 dph larvae. The morphology of the epaxial quadrant was similar on the different sampling days regardless of the treatment group. The myotome consisted of several layers of white muscle fibers covered by a layer of red fibers on the outer edge (Figure 3.8). The red fibers were present up to a certain point on the upper part of the myotome (Figure 3.8B) and at this point the red fibers were positioned more horizontally (Figure 3.8B). The red fibers were smaller than most of the deep white fibers at 9 dph (Figure 3.8C). Generally, the white muscle fibers were bigger right next to the notochord and became smaller outwards from the notochord towards the red fibers in a gradient pattern (Figure 3.8C).

There were smaller white fibers at the apex of the myotome quadrant (Figure 3.8A) and on the inside of the red muscle fibers (Figure 3.8B). This was a stratified hyperplasia pattern which was observed already on 2 dph between the red and white muscle fibers (Figure 3.9B). The recruitment of new white fibers (stratified hyperplasia) occurred along on the inside of the red fiber layer and at the apex of the myotome at 9 and 21 dph, and to some extent at 35 dph. At 21 dph also new recruitments between already existing white fibers (mosaic hyperplasia) was observed for the *Artemia*-fed and cop/cir-fed larvae. This mosaic hyperplasia pattern was observed in the larvae from the different treatments on 35 dph, when the average SL > 8 mm (Figure 3.9D).

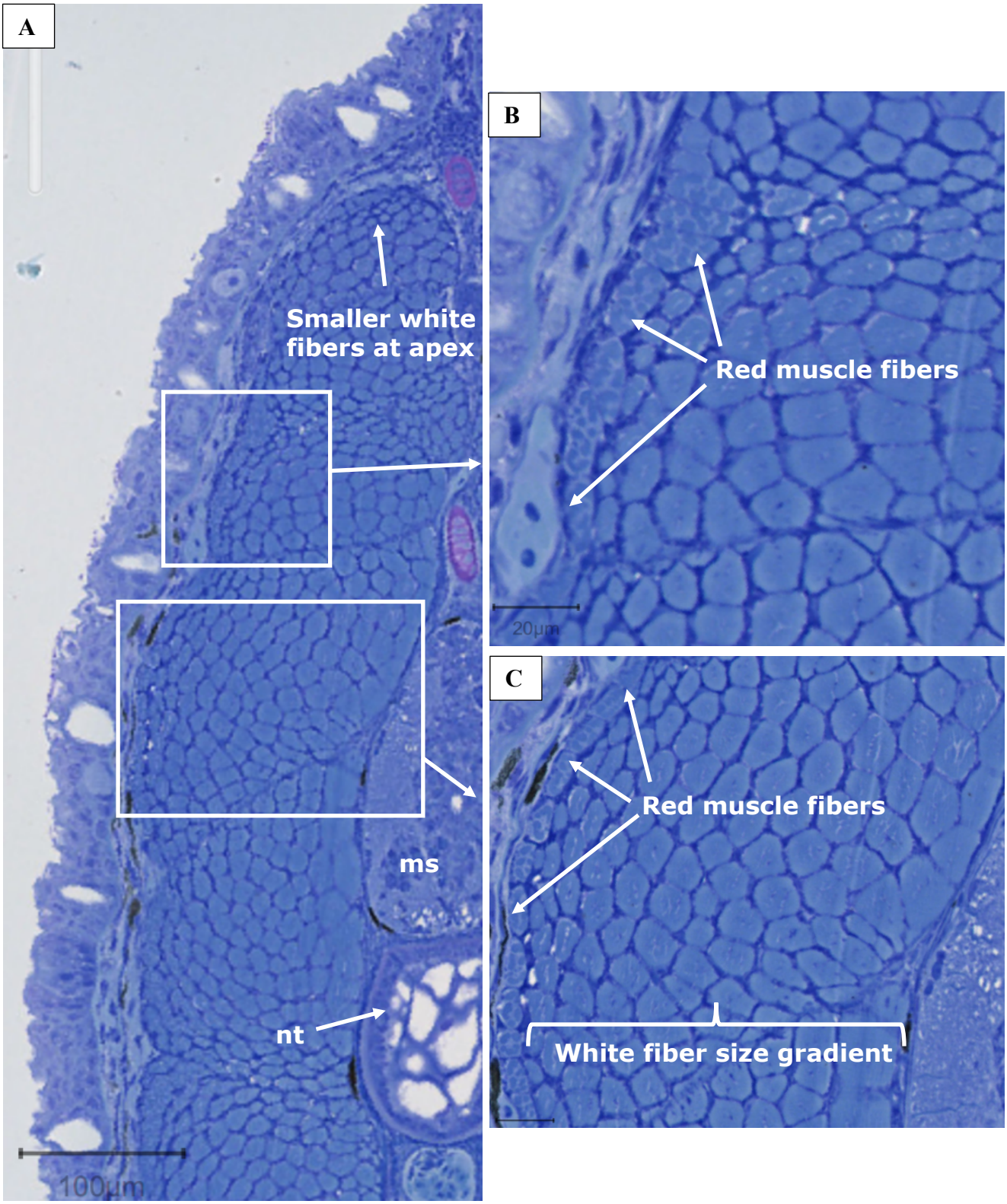


Figure 3.8 Epaxial myotome quadrant of skeletal muscle behind anus for lumpfish larvae. A) The shape of a myotome at 6.3 mm SL (9 dph); B) red muscle fibers in the upper part of the myotome at 6.3 mm SL C) white fiber size gradient in the myotome at 6.3 mm SL. nt, notochord; ms, medulla spinalis; W, white muscle fibers; R, red muscle fibers. Scale bar 100 µm in A and 20 µm in B and C.

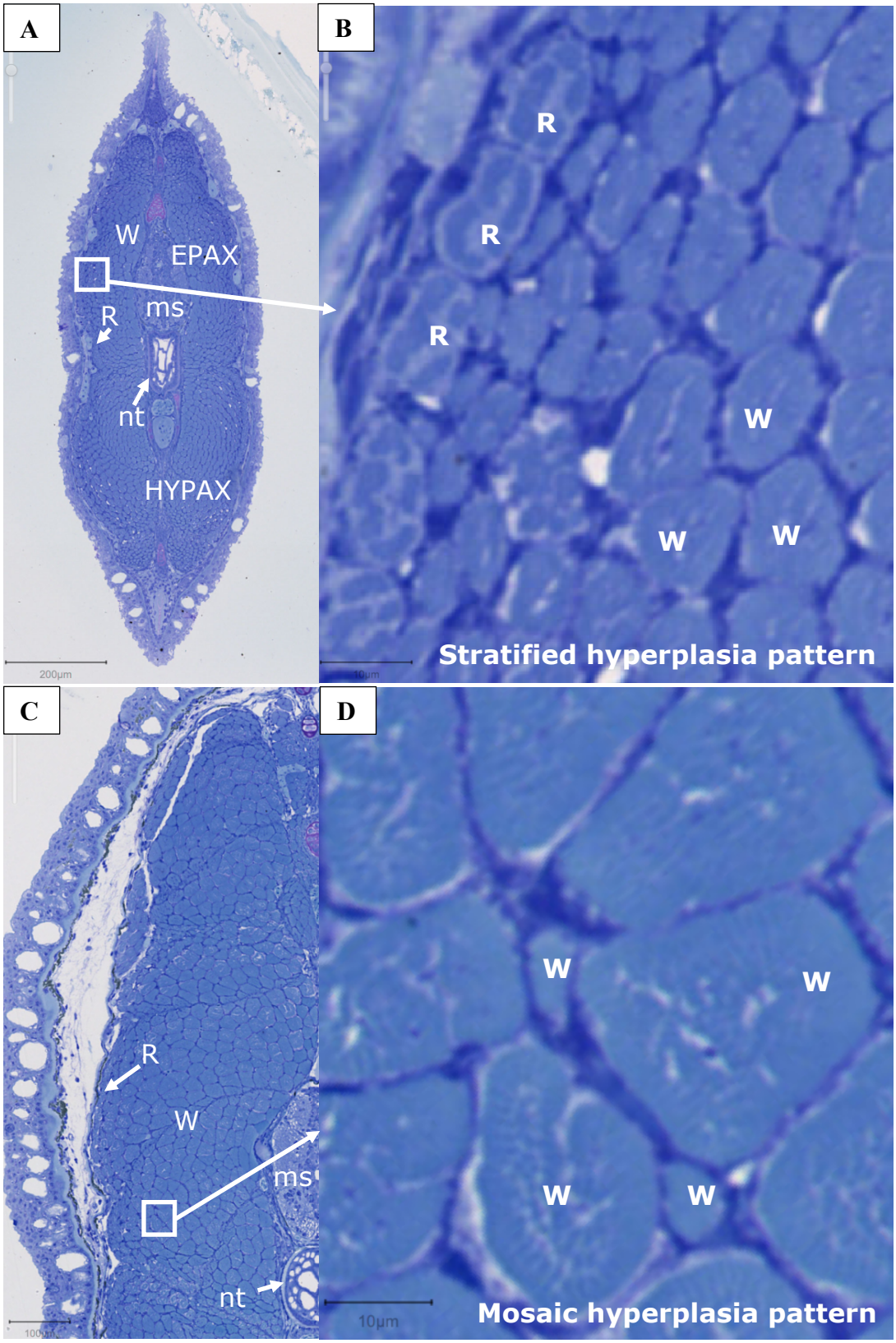


Figure 3.9 Transverse sections of skeletal muscle behind anus in *C.lumpus* larvae. A) The shape of a myotome at 6.2 mm SL (2 dph); B) stratified hyperplasia pattern at 6.2 mm SL, C) the epaxial quadrant of the myotome at 8.5 mm SL (35 dph) and D) mosaic hyperplasia patterns at 8.5 mm SL. EPAX, epaxial quadrant of myotome; HYPAX, hypaxial quadrant of myotome; nt, notochord; ms, medulla spinalis; W, white muscle fibers; R, red muscle fibers. Scale bar 10 µm in B and D 200 µm in A and 100 µm in C.

3.3 Muscle growth in relation to larval age

3.3.1 Red muscle growth

The mean total area of red muscle was 4313 ± 39 μm^2, the mean number of red fibers was 39 ± 2 and the mean size of 30 largest red fibers was 131 ± 4 μm^2 at 2 dph in the quadrant (Table 3.1; Appendix 12-14). No significant differences were found within the larvae between 2 and 9 dph. However, at 9 dph all larvae had a smaller fiber size of the 30 largest fibers, all larvae except the *Artemia*-fed larvae had a smaller total area and the FD-fed larvae had a lower number of fibers at 9 compared to 2 dph (p>0.05). At 21 and 35 dph the total area increased in the larvae fed *Artemia,* cirripedia and cop/cir. At 21 dph the larvae fed cop/FD had a significantly smaller total area and fiber size of the 30 largest fibers compared to 2 dph. At 9 dph, the total area in the larvae fed *Artemia* was significantly larger compared to the larvae from the cop/FD and cop/cir group and larger than cop/FD and FD-fed larvae at 21 dph. The mean red fiber size of the 30 largest fibers was significantly larger compared to the larvae in the cop/cir group at 9 dph. At 35 dph the larvae from the *Artemia* and cop/cir group nearly had a triple-fold increase in the number of red fibers compared to 21 dph and the fiber number was significantly higher compared to the larvae from the cop/FD-group (p<0.05). At the end of the experiment (35 dph) the larvae from the *Artemia* group still attained the highest number of red fibers counting 110 ± 6, nearly double the figure of 59 ± 2 fibers the copepod-fed larvae achieved. The growth of red muscle took place primarily at 35 dph. Only the number of red fibers was significantly different in larvae between the treatments at the end.

Table 3.1 Mean standard length (mm ± SE), total red cross-sectional area (μm^2 ± SE), number of red muscle fibers (n ± SE) and the mean individual size of the 30 largest red fibers (μm^2 ± SE). n = 5 for 2 dph and n = 5 per treatment on 9, 21 and 35 dph. Significant differences (p < 0.05) between treatments on the same day are denoted by different letters.

Dph	Treatment (n =5)	Mean SL (mm)	Total cross-sectional area of red muscle (μm^2)	Number of red muscle fibers (n)	Mean individual size of 30 largest fibers (μm^2)
2	All	5.79 ± 0.13	4313 ± 39	39 ± 2	131 ± 4
9	Art	6.35 ± 0.10	4449 ± 116^a	47 ± 2	124 ± 3^a
	FD	6.22 ± 0.12	3911 ± 184^{ab}	38 ± 2	120 ± 5^{ab}
	Cop/FD	6.22 ± 0.12	3588 ± 314^b	42 ± 2	104 ± 6^{ab}
	Cir	6.34 ± 0.23	3674 ± 184^{ab}	46 ± 6	105 ± 8^{ab}
	Cop/Cir	6.28 ± 0.09	3302 ± 158^b	40 ± 2	98 ± 3^b
21	Art	7.84 ± 0.15^a	5388 ± 618^a	41 ± 3	161 ± 16^a
	FD	6.66 ± 0.15^c	3088 ± 455^b	40 ± 3	96 ± 15^b
	Cop/FD	6.67 ± 0.11^c	2818 ± 315^b	40 ± 2	88 ± 9^b
	Cir	7.52 ± 0.12^{ab}	4926 ± 713^{ab}	45 ± 4	150 ± 19^{ab}
	Cop/Cir	7.16 ± 0.07^b	4238 ± 492^{ab}	40 ± 3	130 ± 12^{ab}
35	Art	9.11 ± 0.27	9467 ± 964	110 ± 6^a	199 ± 22
	FD	8.85 ± 0.29	6935 ± 500	73 ± 2^{ab}	169 ± 10
	Cop/FD	8.13 ± 0.33	5063 ± 357	59 ± 2^b	133 ± 12
	Cir	9.01 ± 0.28	8246 ± 1415	76 ± 11^{ab}	180 ± 20
	Cop/Cir	8.91 ± 0.34	9299 ± 1489	102 ± 5^a	206 ± 29

3.3.2 White muscle growth

The mean total area of white muscle was 41 983 ± 2512 μm^2, the mean number of white muscle fiber was 509 ± 42 and the mean fiber size of the 350 largest white fibers was 107 ± 5 μm^2 on 2 dph (Table 3.2; Appendix 15-17). No significant differences were found within the larvae between 2 and 9 dph. The mean fiber size of the 350 largest fibers and the total white area had increased significantly at 21 dph in all larvae except the ones fed FD and cop/FD (p<0.05). The number of white fibers was significantly higher than at 9 dph in the larvae fed *Artemia*. On 9 dph all larvae were similar and at 21 dph, the larvae in the *Artemia* and cirripedia group had a 3-fold increase and the cop/cir larvae a doubling in total white area and mean fiber size of the 350 largest fibers. The total white area in the *Artemia* and cirripedia larvae was significantly larger compared to the remaining larvae (p<0.050) and the mean fiber size of the 350 largest fibers were larger than the FD and cop/FD larvae. The cop/FD larvae had a significantly lower number of fibers compared to the *Artemia* and Cirripedia larvae at 21 dph. Total white area doubled from 21 to 35 dph in the *Artemia*, cirripedia and cop/cir fed larvae and was significantly higher compared to larvae from the cop/FD group at 35 dph (p<0.05). Mean fiber size of the 350 largest fibers doubled in the larvae fed cop/cir and FD, and at the end of the experiment (35 dph) the larvae in the cop/FD group had a significantly smaller mean fiber size of the 350 largest fibers compared to all the other larvae, except for the FD-fed larvae. The growth of white muscle took place primarily at 21 and 35 dph.

Table 3.2 Mean standard length (mm ± SE), total white cross-sectional area (μm^2 ± SE), number of white muscle fibers (n ± SE) and the mean individual size of the 350 largest white fibers (μm^2 ± SE). n = 5 for 2 dph and n = 5 per treatment on 9, 21 and 35 dph. Significant differences (p< 0.05) between treatments on the same day are denoted by different letters.

Dph	Treatment (n = 5)	Mean SL (mm)	Total cross-sectional area of white muscle (μm^2)	Number of white muscle fibers (n)	Mean individual size of 350 largest fibers (μm^2)
2	All	5.79 ± 0.13	41 983 ± 2512	509 ± 42	107 ± 5
9	Art	6.35 ± 0.10	55 984 ± 5064	587 ± 25	133 ± 10
	FD	6.22 ± 0.12	50 185 ± 4466	524 ± 35	125 ± 11
	Cop/FD	6.22 ± 0.12	62 082 ± 4666	623 ± 26	144 ± 10
	Cir	6.34 ± 0.23	54 826 ± 5151	616 ± 74	132 ± 15
	Cop/Cir	6.28 ± 0.09	57 802 ± 4188	597 ± 21	136 ± 8
21	Art	7.84 ± 0.15^a	175 370 ± 11 801^a	744 ± 34^a	372 ± 16^a
	FD	6.66 ± 0.15^c	77 572 ± 12 759^b	604 ± 29^{ab}	186 ± 30^b
	Cop/FD	6.67 ± 0.11^c	89 490 ± 11 599^b	568 ± 42^b	221 ± 27^b
	Cir	7.52 ± 0.12^{ab}	171 196 ± 12 602^a	742 ± 18^a	369 ± 25^a
	Cop/Cir	7.16 ± 0.07^b	115 976 ± 7818^b	632 ± 40^{ab}	280 ± 11^{ab}
35	Art	9.11 ± 0.27	278 715 ± 13 000^a	843 ± 19	588 ± 29^{ac}
	FD	8.85 ± 0.29	196 937 ± 25 046^{ab}	740 ± 43	433 ± 42^{bc}
	Cop/FD	8.13 ± 0.33	159 300 ± 13 599^b	751 ± 10	350 ± 31^b
	Cir	9.01 ± 0.28	273 627 ± 30 472^a	803 ± 22	582 ± 57^{ac}
	Cop/Cir	8.91 ± 0.34	285 185 ± 24 215^a	792 ± 17	611 ± 41^a

3.3.3 White and red muscle growth

The total area on 2 dph of red and white muscle area combined was 46 296 ± 2621 μm^2, from which white and red muscle constituted 90.7% and 9.3% respectively (Table 3.3). Throughout the experimental period the percentage of white muscle of the total area increased, until the weaning period (21 dph) started. At 21 and 35 dph, the percentage of white and red muscle remained stable, and no differences were observed between the larvae in the different treatments when the experiment was terminated (35 dph). At 9 dph, the larvae in all the treatment groups attained a similar total area of white and red muscle combined. On the following sampling day (21 dph), the total area of the *Artemia*-fed and cirripedia-fed larvae had increased by a 3-fold and the total area was significantly higher compared to the larvae in the three remaining groups although they increased by a 2-fold (p<0.05). The percentage of white muscle in all the larvae increased by 3-4 %, whereas in the red muscle it decreased with 3-4% from 9 to 21 dph. At 35 dph the cop/cir and FD-fed larvae's total area had increased by a 2-fold. At 35 dph, the larvae in the cop/cir, *Artemia* and cirripedia group obtained a significantly bigger total area of red and white muscle compared to the cop/FD group (p<0.05). Where the larvae in the cop/cir had a slightly bigger total area, whereas the cirripedia-fed larvae had the highest % of white muscle.

Table 3.3 Mean standard length (mm ± SE), total cross-sectional area (μm^2) and percentage (%) of red and white muscle in *C.lumpus* from 2-35 dph. n=5 on 2 dph, and n=5 per treatment on 9, 21 and 35 dph. Error bars are ± standard error (SE) and significant differences (p< 0.05) between the treatments on the same day are indicated with different letters.

Dph	Treatment	Mean SL (mm)	Total area of white and red muscle (μm^2)	% Of white muscle of total area	% Of red muscle of total area
2	All	5.79 ± 0.13	46 296 ± 2621	90.7	9.3
9	Art	6.35 ± 0.10	60 434 ± 5154	92.6	7.4
	FD	6.22 ± 0.12	54 096 ± 4641	92.8	7.2
	Cop/FD	6.22 ± 0.12	65 670 ± 4912	94.5	5.5
	Cir	6.34 ± 0.23	58 499 ± 5121	93.7	6.3
	Cop/Cir	6.28 ± 0.09	61 103 ± 4050	94.6	5.4
21	Art	7.84 ± 0.15^a	180 758 ± 12 278^a	97.0	3.0
	FD	6.66 ± 0.15^c	80 659 ± 13 125^b	96.2	3.8
	Cop/FD	6.67 ± 0.11^c	92 308 ± 11 852^b	96.9	3.1
	Cir	7.52 ± 0.12^{ab}	176 122 ± 13 257^a	97.2	2.8
	Cop/Cir	7.16 ± 0.07^b	120 214 ± 8098^b	96.5	3.5
35	Art	9.11 ± 0.27	288 182 ± 13 017^a	96.7	3.3
	FD	8.85 ± 0.29	203 872 ± 25 499^{ab}	96.6	3.4
	Cop/FD	8.13 ± 0.33	164 364 ± 13 762^b	96.9	3.1
	Cir	9.01 ± 0.28	281 874 ± 31 758^a	97.1	2.9
	Cop/Cir	8.91 ± 0.34	294 485 ± 25 584^a	96.8	3.2

3.4 Muscle fiber growth in relation to larval size

The total area of both red and white larval muscle increased exponentially with the standard length of the *C.lumpus* larvae from around 6.5 mm SL regardless of the treatment group (Figure 3.10). From the lowest (5.5 mm) to the highest (10 mm) SL, the total white area increased by a 7-fold, whereas the red total area increased by a 6-fold. The percentage of red muscle (of the total area of both red and white muscle) decreased over time (Table 3.3) and exponentially when correlated to the SL (r^2=0.926, P<0.001) (Figure 3.10). Red muscle constituted approximately 9 % of the white and red total area at 6 mm SL and the percentage decreased exponentially with increasing SL down to 3% in larvae at 7.5 mm SL and remained stable at 3% after that (Figure 3.10).

The fiber number and the mean fiber size of red and white muscle in the larvae increased exponentially with the standard length of *C.lumpus* larvae regardless of the treatment group (Figure 3.11). From the initial SL 5.5 mm until the end of the experiment at 10 mm SL the number of red fibers in the larvae increased by a 4-fold, and the mean fiber size doubled. The number of white fibers doubled, and the mean fiber size increased by an 8-fold.

The mean number of fibers and the mean fiber size of red and white muscle were strongly correlated to SL (Figure 3.11) and was confirmed with a one-way ANCOVA (Table 3.4 and Table 3.5) with P<0.0001.

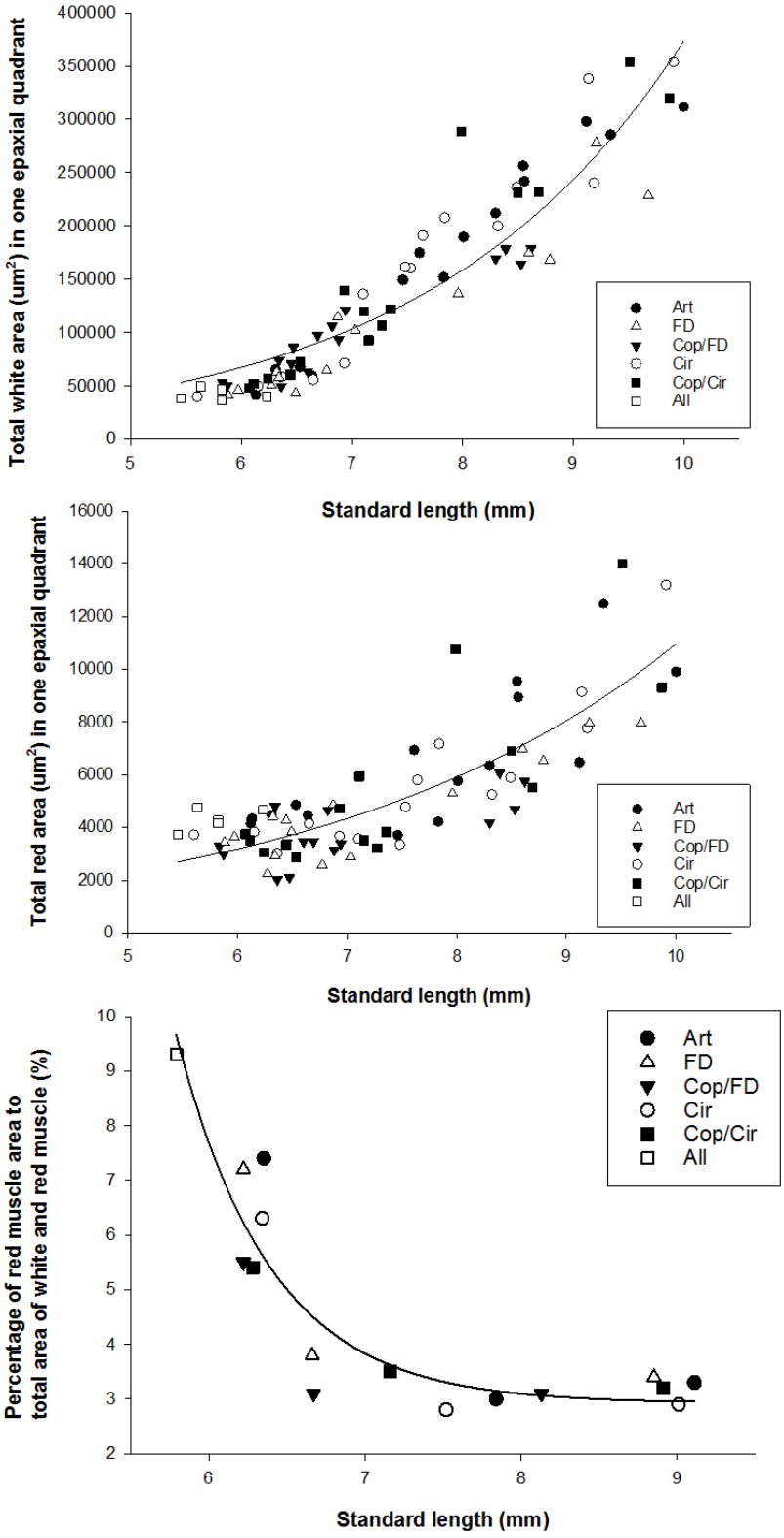


Figure 3.10 Total white and red area in *C.lumpus* in one epaxial quadrant of the myotome and the percentage of red muscle to total area of white and red muscle (%). Each point in the graph represents one larva from 2 (*n*=5), 9, 21 and 35 dph (*n*=5 per treatment). Exponential curve was the best fit. r^2= 0.858, r^2= 0.678 and r^2= 0.926 respectively.

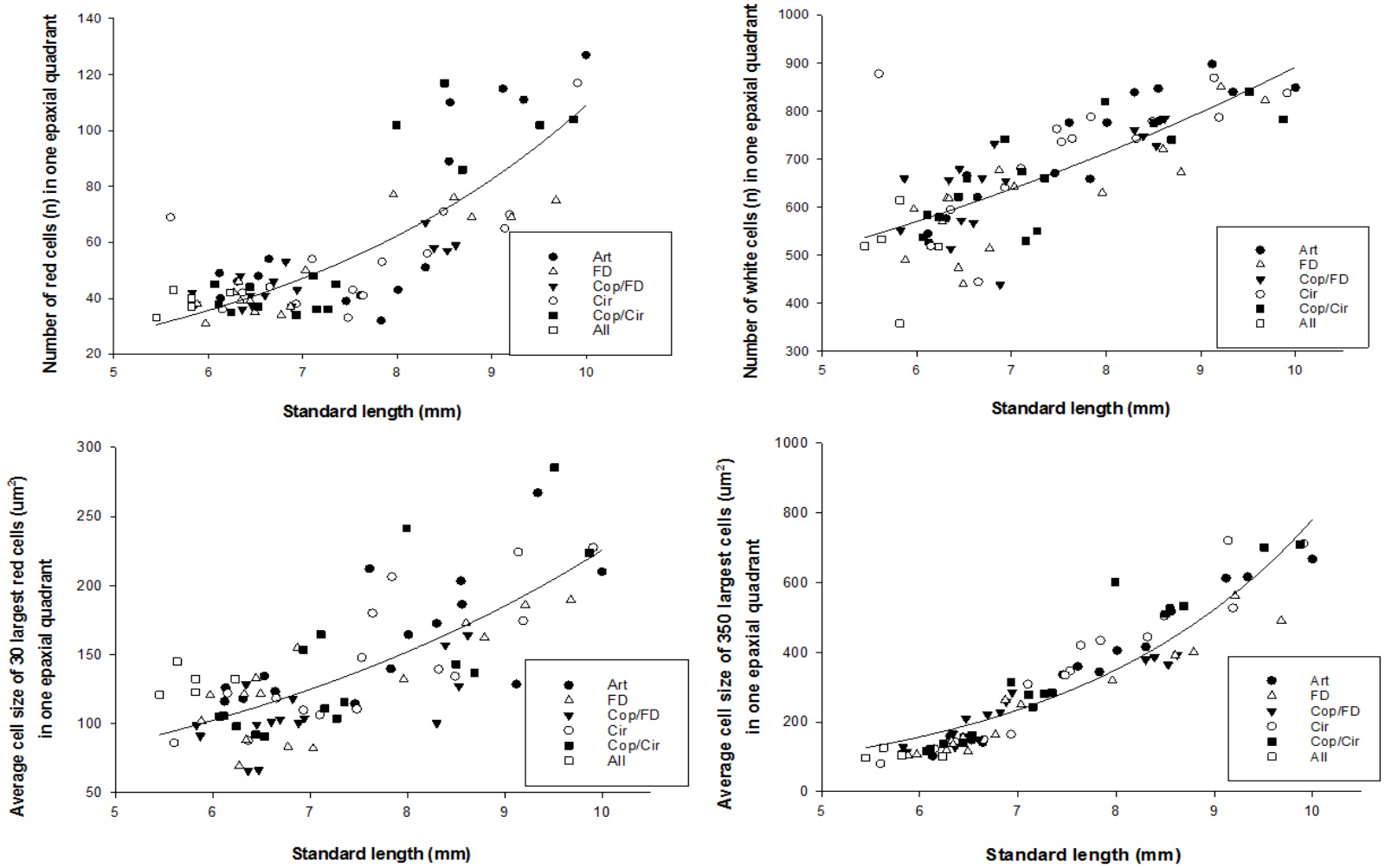


Figure 3.11 The number of fibers and the mean fiber size of the 30 and 350 largest red and white fibers in *C.lumpus* in one epaxial quadrant of the myotome. Each point in the graph represents one larva from 2 (*n*=5), 9, 21 and 35 dph (*n*=5 per treatment). Exponential growth lines were added. r^2 =0.655 for the number of red fibers and r^2=0.556 for the mean fiber size of the 30 largest fibers in red muscle. r^2 =0.581 for the number of white fibers and r^2=0.872 for the mean fiber size of the 350 largest fibers in white muscle.

Table 3.4 Variance ratio and probability value of one-way ANCOVA analysis for red muscle.

	Nr. Of red fibers (n)		**Total red area (μm²)**		**Mean individual size of 30 largest red fibers (μm²)**	
Source	***F***	***P***	***F***	***P***	***F***	***P***
Treatment	1.617	0.177	0.649	0.629	1.019	0.402
SL	156.139	**<0.001**	92.336	**<0.001**	60.784	**<0.001**
Treatment x SL	1.955	0.108	0.964	0.431	1.467	0.219

Table 3.5 Variance ratio and probability value of one-way ANCOVA analysis for white muscle.

	Nr. Of white fibers (n)		**Total white area (μm²)**		**Mean individual size of 350 largest white fibers (μm²)**	
Source	***F***	***P***	***F***	***P***	***F***	***P***
Treatment	0.264	0.900	0.731	0.573	0.860	0.491
SL	100.889	**<0.001**	850.378	**<0.001**	905.321	**<0.001**
Treatment x SL	0.361	0.836	1.082	0.370	1.223	0.307

3.5 Mosaic hyperplasia growth

The mosaic hyperplasia pattern was observed in the myotome of larvae from all the different treatment groups on 35 dph, when the average SL > 8 mm (Figure 3.9D, Figure 3.12). From 5.5 mm until 8 mm SL (2-21 dph) either one or none mosaic fibers was observed in all the larvae. The highest number of white mosaic fibers was 40 in an *Artemia* fed larva at approximately 9.5 mm SL (Figure 3.12).

On the day the experiment was terminated (35 dph), the larvae from the *Artemia* group had a significantly higher total number of white mosaic fibers compared to the larvae in the remaining treatment groups ($p<0.05$) (Table 3.6). On 35 dph the number of mosaic fibers were significantly correlated to SL ($p=0.010$), but not to the treatment group ($p=0.714$) (Table 3.7).

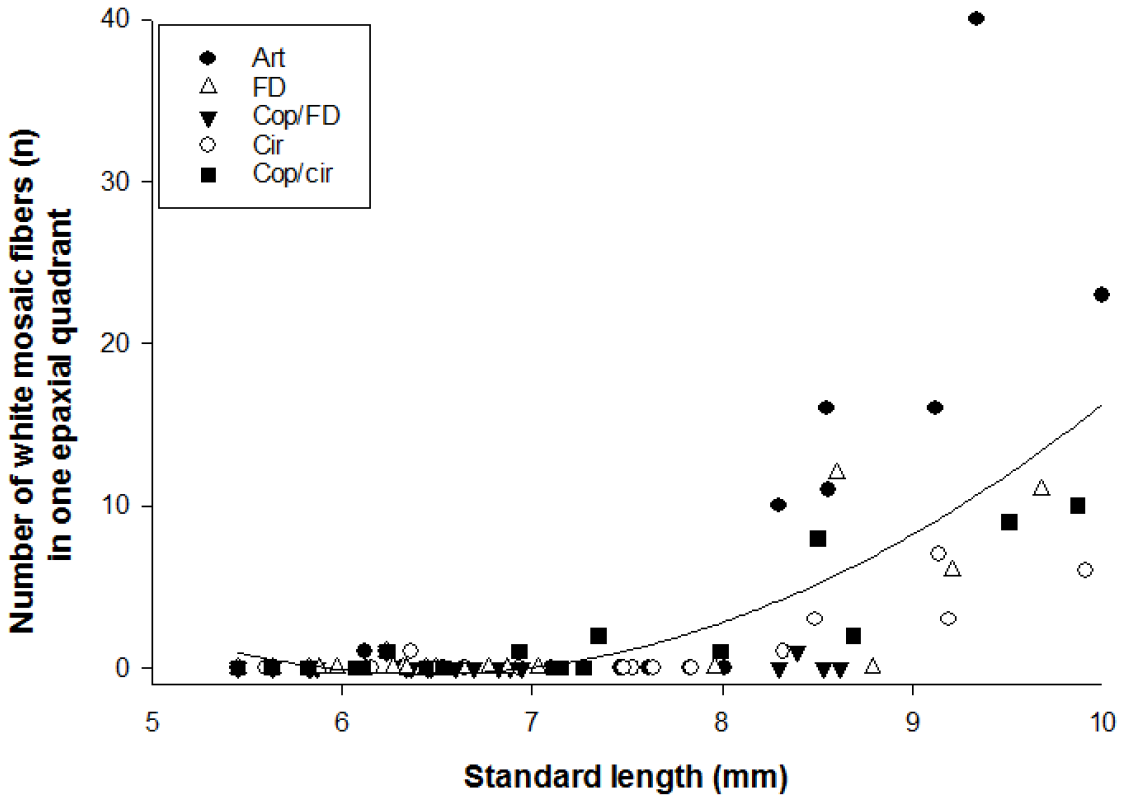


Figure 3.12 Total number of white mosaic fibers in one epaxial quadrant of the myotome in individual *C.lumpus* larva. *n*=20 per treatment. A global curve fit was added, where polynomial quadratic fit was the best (r^2=0.70, *P*<0.0001).

Table 3.6 Total number of mosaic hyperplasia fibers (n) in *n*=5 *C.lumpus* larvae in each treatment group from 2 to 35 dph. Error bars are ± standard error (SE) and significant differences (*p*< 0.05) on 35 dph between the treatments are indicated with different letters.

Dph	Treatment (*n* = 5)	Mean SL (mm)	Total number of white mosaic fibers (n)
2	All	5.79 ± 0.13	1 ± 0
9	Art	6.35 ± 0.10	1 ± 0
	FD	6.22 ± 0.12	0 ± 0
	Cop/FD	6.22 ± 0.12	0 ± 0
	Cir	6.34 ± 0.23	1 ± 0
	Cop/Cir	6.28 ± 0.09	1 ± 0
21	Art	7.84 ± 0.15^{a}	10 ± 2
	FD	6.66 ± 0.15^{c}	0 ± 0
	Cop/FD	6.67 ± 0.11^{c}	0 ± 0
	Cir	7.52 ± 0.12^{ab}	0 ± 0
	Cop/Cir	7.16 ± 0.07^{b}	3 ± 0
35	Art	9.11 ± 0.27	106 ± 5^{a}
	FD	8.85 ± 0.29	29 ± 3^{bc}
	Cop/FD	8.13 ± 0.33	1 ± 0^{b}
	Cir	9.01 ± 0.28	20 ± 1^{bc}
	Cop/Cir	8.91 ± 0.34	30 ± 2^{c}

Table 3.7 Variance ratio and probability value of one-way ANCOVA analysis for mosaic white fibers on 35 dph.

	Nr. Of mosaic fibers on 35 dph (*n*)	
Source	***F***	***P***
Treatment	0.533	0.714
SL	8.644	**0.010**
Treatment x SL	0.667	0.625

4 Discussion

4.1 Larval growth and survival in relation to feeding regime and diet

Feeding with enriched *Artemia* nauplii provided the highest lumpfish larval growth and survival, and the growth was significantly higher at 15 dph and to the end of the experiment (35 dph). Feeding with *Artemia* in lumpfish larvae have improved growth in other lumpfish studies too (Marthinsen et al., 2018, Hanssen et al., 2018, Rian et al., 2019). The *Artemia* nauplii might be preferred because of its large size compared to the other feed types used in the present study (Baert et al., 1996). Larger prey is easier to detect and can provide a higher available biomass and a higher consumption could be obtained with less energy used (Killen et al., 2007). Furthermore, the pace of the *Artemia* nauplii is relatively slow and might therefore be suitable for the lumpfish larvae since it usually attaches to surfaces and has a poor maximum swimming speed and a relatively low aerobic scope (Hvas et al., 2018). That means less aerobic energy is available for activities like locomotion and foraging and a passive foraging mode can therefore be preferable to save energy for growth instead (Hvas et al., 2018). The *Artemia*-fed larvae had a high DWI compared to the other regimes in the live prey period, and a lower DWI in the period when the weaning started (21 dph) and till the experiment ended (35 dph). The lower growth at weaning might be because the weaning itself is stressful (Powell et al., 2018b), but the overall survival is still high at that time in the *Artemia*-fed larvae. The transition to a much smaller feed (Gemma micro 300) might be challenging as the larvae usually consume larger feed as it grows (Yúfera, 2011). Besides, the FD only float with the water current and might not stimulate the predatory behavior in the same extent as the *Artemia* nauplii (D'Abramo, 2002). Still, the larvae fed cirripedia nauplii had a higher DWI after weaning at 21 dph compared to the DWI in the live feed period. Therefore, the predatory behavior may not be the primary cause for the lower DWI at weaning in the larvae fed *Artemia*.

The larvae fed cirripedia nauplii had the second highest growth at the end of the experiment and the main growth increase occurred at weaning to FD at 21 dph. The cirripedia nauplii is smaller than the enriched *Artemia* nauplii, but bigger than the copepods and the smallest FD-type (Baert et al., 1996, C-FEED, 2014, Planktonic, 2021, Skretting, 2021). The cirripedia-fed larvae had the lowest DW (9 dph) and a negative DWI trend (2-9 dph) and might therefore only have had precisely enough energy to grow minimally and survive, but possibly not in all the larvae. Feeding with cop/FD resulted in the poorest larval growth, significantly lower than the *Artemia* and cirripedia group. Other lumpfish studies report similar observations (Marthinsen et al., 2018, Hanssen et al., 2018, Rian et al., 2019), whereas in the pelagic species ballan wrasse, cod and sea bass larvae, feeding with copepods and rotifers improved growth rates (Berg et al., 2012, Romundstad, 2015, Rajkumar and Kumaraguru vasagam, 2006, Øie et al., 2015). Common for the pelagic species is their small size at hatching compared to the lumpfish

larvae. The lumpfish hatch from demersal eggs with a longer developmental time from fertilization to hatching, compared to larvae from pelagic eggs (Kjørsvik et al., 2004). Larger eggs contain more yolk and energy, and provide larger fish larvae which can develop a better digestive system, functional mouth and eyes to use at hatching to eat larger prey (Kjørsvik et al., 2004). The larger size of the *Artemia* (Baert et al., 1996) and cirripedia (Planktonic, 2018) could thus be a better fit for a more developed lumpfish, compared to the smaller pelagic fish larvae. A bigger prey size provides a higher biomass available and if the larvae are given a feed which is of the right size according to its mouth size, a higher consumption could be obtained with less energy used (Killen et al., 2007). Furthermore, the copepod swims relatively fast, and since the lumpfish larvae have a poor maximum swimming speed and a relatively low aerobic scope (Hvas et al., 2018) the larvae might have to use considerable amount of energy to catch copepods.

Generally, fish larvae have a high growth potential when provided a diet of the right quality and quantity and that includes high amounts of the PUFAs DHA and EPA (Navarro et al., 1999, Moksness et al., 2008). Of the total fatty acid in *Artemia* and cirripedia nauplii, 22% of them are DHA (Øie et al., 2015, Planktonic, 2021). In the cirripedia nauplii 25% are EPA, whereas 5.3% are EPA in *Artemia* nauplii (Øie et al., 2015, Planktonic, 2021). In comparison, the copepods have 13% DHA and 11% EPA (Øie et al., 2015). In the copepod and cirripedia nauplii the DHA and EPA are located primarily in the phospholipids (Albers et al., 1996, Tokle, 2021). This location provides a more effective utilization for larval growth (Gisbert et al., 2005, Wold et al., 2007, Kjørsvik et al., 2009, Wold et al., 2009) compared to PUFAs located in the neutral lipids like the enriched *Artemia* nauplii have (Conceição et al., 2010). Another aspect related to the dietary quality is the proteins and amino acids which can affect both the growth and survival (Øie et al., 2015). The protein amount is approximately the same in the cirripedia and copepod nauplii (67g/ 100g DW) and almost double the amount compared to the proteins in *Artemia* nauplii (35g/100g DW) (Øie et al., 2015, Planktonic, 2021). Still, the highest larval growth and survival at the end of the experiment was in the *Artemia*-fed larvae. Hence, the protein might not limit the larval growth and the location of the fatty acids might not be that crucial for effective utilization in the lumpfish larvae.

Feeding with the cop/cir group increased larval growth rate after the first dietary change from copepod to cirripedia but obtained slow growth after the second change to Gemma micro 300. In Marthinsen et al. (2018) and Hanssen et al. (2018), a slower growth was obtained after the first dietary change from copepods to formulated diet and thereafter an increased growth after changing to a bigger FD size. In cod larvae, a dietary change affected the growth rate positively when the diet was changed from enriched rotifers to copepods, but not the other way around (Koedijk et al., 2010). The size of the feed probably contributed to the changing growth rates, as copepods are bigger than rotifers (Koedijk et al., 2010), same as cirripedia is compared to *A.tonsa* in the present study (Planktonic, 2018, C-FEED, 2014). The size of the feed matters as the fish larvae grows bigger it usually consumes larger feed (Yúfera, 2011). Moreover, the weaning itself is a critical point in the rearing of lumpfish as it stresses the larva (Powell et al., 2018b). In this feeding regime there were two weaning periods, and it might therefore have been extra stressful for the larvae and less energy might have been put into growth.

The larvae in the cop/cir and cop/FD group were both fed copepods before weaning at 10 dph and had similar larval SL, DW and DWI. The growth in the cop/cir larvae increased after the first dietary change from copepod to cirripedia and had a significantly higher SL

and DW and a higher survival compared to the cop/FD larvae weaned to Gemma micro 150 and 300 µm. The different growth after weaning might be due to the size of the feed (Yúfera, 2011) as the larvae in the cop/cir group received a larger prey whereas the larvae in the cop/FD group received FD-pellets of both 150 and 300 µm. The size of the feed can play a crucial role when the transition to exogenous feeding starts and thus affect the feed intake as smaller food items might be harder to find (Hunter, 1981), even though the lumpfish larva acquire functional eyes already at hatching (Brown, 1986). The larvae fed exclusively with FD had a similar growth rate as the cop/FD group and Gemma micro 150 (Skretting, 2021) used in the beginning of the FD feeding regime was similar in size as the copepod (C-FEED, 2014) but smaller than the *Artemia* and cirripedia (Baert et al., 1996, Planktonic, 2021). It might seem like the copepod and the smallest formulated diet (150 µm) were too small for the lumpfish larvae at hatching. The ingestion rate in the FD group and in the cop/cir group (after weaning to FD) might be lower since the FD-pellets drift with the water currents and not actively swims. Hence it might not trigger the predatory behavior in the larvae to the same extent as with live feeds (D'Abramo, 2002). The digestion of the formulated diet might have been limited by the polymerization of proteins used to prevent leaching (Nordgreen et al., 2008), as the functional stomach of lumpfish is not fully differentiated until 34 dph (Marthinsen et al., 2018). This indicates that the use of formulated pellets in the start of the exogenous feeding might not be optimal considering the absence of a fully functional digestive system in lumpfish larvae.

The mean survival in all the feeding regimes was over 85%, and other studies also reports high survival in cultivated lumpfish larvae (Marthinsen et al., 2018, Hanssen et al., 2018, Dahle et al., 2017). Increased mortality was observed at approximately 21 dph in all the larvae, and the cop/FD larvae had the highest mortality. This might have been due to overfeeding as the cop/FD larvae were smaller compared to the rest of the larvae at that time but were fed the same amount of FD. The cop/FD-fed larvae had the largest drop in survival from 92 to 85% and this was also observed in Hanssen et al. (2018), Marthinsen et al. (2018), Rian et al. (2019) and Dahle et al. (2017). Still, the overall survival rate in lumpfish larviculture is significantly higher than in pelagic species like ballan wrasse (Berg et al., 2012, Romundstad, 2015, Øie et al., 2015) and Atlantic cod (Øie et al., 2015).

Based on the present findings, the nutritional requirements for the lumpfish larvae at start-feeding is still not completely identified. Feeding with *Artemia* to the lumpfish larvae has resulted in better growth and survival in the present study and in several other start feeding studies (Marthinsen et al., 2018, Hanssen et al., 2018, Rian et al., 2019). Feeding with copepod seems to be more suitable for smaller pelagic fish species (Conceição et al., 2010). While, formulated diet might be the a better option for larger fish species, since the stomach of lumpfish larvae is not fully differentiated until 34 dph (Marthinsen et al., 2018). The best option for this intermediate sized fish might possibly be to start feeding on *Artemia* nauplii and wean over to a larger FD size possibly after the stomach is fully differentiated (34 dph) as that can increase the larval protein digestion capacity (Marthinsen et al., 2018). In the present study, weaning to FD occurred at two different times (10 and 21 dph) and a later weaning seems to be more ideal based on the growth, but perhaps the weaning at 10 dph could have been improved by using a larger FD. Since the lumpfish seems to accept and prefer large preys already at hatching. Further research on start-feeding with lumpfish should include feeding regimes with cirripedia nauplii as it provided high larval growth in the present study and is a relatively

new prey. Another interesting feeding regime could consist exclusively with FD but with a larger FD size than in the present study from start.

4.2 Muscle growth and development in relation to feeding regimes

In lumpfish larvae, it seems like stratified hyperplasia muscle growth started already in the embryonic stage before hatching since the white fibers were in a gradient pattern and small white fibers were observed along the inside of the red fiber layer already at 2 dph. This is before the exogenous feeding started (2 dph). The mosaic hyperplasia growth was observed in all the different treatment groups at 35 dph (8-9 mm SL) in late larval/early juvenile stages. In other marine fish species such as ballan wrasse (Berg et al., 2012), cod (Galloway et al., 1999), European sea bass (Veggetti et al., 1990) and gilthead sea bream larvae (Rowlerson et al., 1995), the stratified and mosaic hyperplasia mostly starts at the onset of exogenous feeding and in late larval/early juvenile stages respectively. In Atlantic salmon and common whitefish (*Coregonus lavaretur*) (Stickland et al., 1988, Steinbacher et al., 2017) the stratified hyperplasia also starts in the late embryonic stages and the mosaic hyperplasia mostly starts at the onset of exogenous feeding. The lumpfish larvae might therefore seem to hatch at an enhanced developmental stage compared to the pelagic larval species since they often require an exogenous feed source to start stratified hyperplasia (Galloway et al., 1999, Veggetti et al., 1990, Rowlerson et al., 1995). This corresponds to the larval growth results which endorses that the lumpfish larvae seem to be better developed at hatching compared to the pelagic species since lumpfish larvae appears to prefer bigger preys already at hatching. The mosaic hyperplasia pattern is normally present from early juvenile stage and throughout large parts of the fish's life span (Weatherley et al., 1988, Johnston, 2006). The mosaic hyperplasia in lumpfish was mostly present at 35 dph (8-9 mm SL), and this corresponds well with the appearance of a functional stomach and pyloric caeca in lumpfish at 34 dph (8.0 - 9.1 mm SL) (Marthinsen et al., 2018). Respectively, these appearances can be used to indicate the climax-metamorphosis period in teleost larvae (Tanaka, 1971, Govoni et al., 1986, BISBAL and Bengtson, 1995). The mosaic hyperplastic growth in cod was found from the post-climax metamorphosis (22 mm SL) (Vo et al., 2016). Mosaic hyperplasia was correlated significantly to the SL when the lumpfish larvae were > 8 mm (SL).

The present study found a strong correlation between muscle growth (hyperplasia, hypertrophy, total cross-sectional area, and mosaic hyperplasia) and larval body size (SL). A similar correlation was found in larvae of cod (Vo et al., 2016), ballan wrasse (Berg et al., 2012), *Clarias gariepinus* larvae (Akster et al., 1995) and in juvenile Atlantic salmon (*Salmo salar*) (Higgins and Thorpe, 1990). This indicates that muscle growth in lumpfish larvae is more related to the body length rather than the larval age. The red muscle grew mainly by hypertrophy from 6-8 mm SL, and mainly by hyperplasia in 8-9 mm SL. Whereas the white muscle grew by both hypertrophy and stratified and mosaic hyperplasia in 6-9 mm SL. Generally, fish muscle hypertrophy occurs continuously while hyperplasia occurs in different waves (Weatherley et al., 1988, Johnston, 1999). Muscle growth dynamics between hypertrophy and hyperplasia at certain body sizes were demonstrated in cod larvae and were associated with transitional metamorphic stages (Vo et al., 2016). In red muscle, hypertrophy occurred at all times and hyperplasia

between 10-20 mm SL, whereas in white muscle the growth was predominated by hypertrophy (4-7 mm SL), and then hyperplasia (6-12 mm SL). Both growth mechanisms occurred from 10-15 mm SL (Vo et al., 2016).

An increased hyperplastic growth was observed correspondingly with an increased somatic growth rate from 9 dph in lumpfish. The hyperplastic growth appears to increase in accordance with the somatic growth in several teleost species such as cod (Galloway et al., 1999), muskellunge (Weatherley et al., 1988), rainbow trout (Weatherley et al., 1988), Atlantic salmon (Higgins and Thorpe, 1990), Atlantic herring (Johnston et al., 1998), pike perch (Ostaszewska et al., 2008) and carp (*Cyprinus carpio*) (Alami-Durante et al., 1997). The growth potential in juvenile and adult fish is thought to be improved by the increased somatic- and hyperplastic growth rate in the early stages (Galloway et al., 1999, Ostaszewska et al., 2008, Valente et al., 2013). Since the muscle tissue is the most rapidly growing tissue in fish (Osse and Van den Boogaart, 1995), environmental effects on larval growth usually also affects the muscle growth in hyperplasia or hypertrophy. Live preys and their composition can affect the larval growth as well as the muscle growth and have been reported in cod and ballan wrasse where feeding with copepod nauplii provided a significantly higher somatic growth rate corresponding with a higher hypertrophic and hyperplastic growth in comparison to rotifer nauplii (Vo et al., 2016, Berg et al., 2012).

The effects of feeding type and regime on the muscle growth and body size growth in lumpfish were mostly found at 21 dph. At 21 dph the red muscle had increased in mean fiber size, except for the cop/FD and FD-fed larvae which had a significantly lower mean fiber size (of the 30 largest fibers) compared to 2 dph. The muscle growth might therefore have been negatively affected by the diet. Whereas for the white muscle, the mean fiber size of the 350 largest fibers had increased by a 2- and 3-fold and the larvae fed *Artemia* and cirripedia had a significantly larger mean fiber size. Hypertrophic growth in white muscle and hyperplastic growth in red muscle of the larvae were significantly affected by the feed type at the end of the experiment (35 dph). *Artemia* and cop/cir larvae had a higher number of red fibers, whereas in the white muscle the fiber size in the larva fed *Artemia*, cirripedia and cop/cir was significantly larger than in the cop/FD-fed larvae. In ballan wrasse the larvae fed copepod had the biggest fiber size and a significantly higher number of white fibers at the end (Berg et al., 2012). After having the same formulated diet for approximately two weeks in the cop/FD (and FD group), a negative effect in the red muscle hyperplasia and white muscle hypertrophy was observed and this corresponds to the larval growth results where cop/FD (and FD) provides the lowest growth. It therefore seems like feeding with copepod is not optimal in lumpfish larvae for either larval or muscle growth even if it provides good results in other species.

Since stratified hyperplasia started in the embryonic stage in the lumpfish, the exogenous feed might not have had the same effect on the muscle growth as in the pelagic species which requires exogenous feed (Galloway et al., 1999, Berg et al., 2012, Veggetti et al., 1990, Rowlerson et al., 1995). However, the further stratified hyperplasia growth in lumpfish requires exogenous feed. Copepods seems to be the poorest- and *Artemia* the best diet for both somatic- and muscle growth in lumpfish larvae. Therefore, further start-feeding should rather focus on providing the bigger live preys or a mix. A greater muscle mass and development could increase the swimming capacity and thereby make the lumpfish larvae better suited for capturing prey and grow bigger.

5 Conclusion

The present study demonstrated that using enriched *Artemia* nauplii in the start-feeding of lumpfish larvae enhanced the somatic growth rate compared to feeding with copepods and formulated diet. Feeding with cirripedia nauplii provided a similar growth as in the larvae fed *Artemia* at the end of the experiment. A higher growth and survival as seen in the larvae fed *Artemia* can provide a more predictable and stable larvae cultivation. Feeding with copepod seems to be more suitable for smaller pelagic fish species, whereas formulated diet might be a better option for larger fish species, since the stomach of lumpfish larvae is not fully differentiated early in the start-feeding. Based on the present findings, the nutritional requirements for the lumpfish larvae at start-feeding is still not completely identified but the best option for this intermediate sized fish might be to start feeding with *Artemia*, before weaning over to larger formulated pellet after the stomach is fully differentiated. However, more research is needed on start-feeding with cirripedia nauplii as this is a relatively new live prey.

Furthermore, at the end of the experiment (35 dph) feeding with *Artemia* and the mixed regime cop/cir increased hyperplastic growth in red muscle and feeding with *Artemia*, cirripedia and the cop/cir group had an increased growth effect on hypertrophy in white muscle of the larvae in comparisons with feeding cop/FD at 35 dph. Feeding with *Artemia* provided a higher number of mosaic fibers at the end of the experiment and it appeared earlier in time. The present study found a strong correlation between muscle growth (hyperplasia, hypertrophy, total cross-sectional area, and mosaic hyperplasia) and larval body size (SL). This indicates that muscle growth in lumpfish larvae is more related to the body length rather than the larval age. Stratified hyperplasia was observed at 2 dph and had possibly started already in the embryonic stage. Stratified hyperplastic growth continued at 9 and 21 dph and to some extent at 35 dph, when the mosaic hyperplastic growth started. After hatching the larval red muscle grew by hypertrophy from 6-8 mm SL and mainly by hyperplasia in 8-9 mm SL. The white lumpfish muscle grew by both stratified and mosaic hyperplasia and hypertrophy from 6-9 mm SL. The mosaic hyperplastic growth was found to correlate to the climax-metamorphosis period in lumpfish at 8.1 – 9.1 mm SL (35 dph).

To optimize rearing protocols for the lumpfish the present findings provide useful information about how the larvae grow on the different diets and how the muscle develops and grow based on the start-feed. The larger preys seem to be more optimal for the lumpfish larvae already after hatching. Further research should aim to look more into start-feeding with cirripedia nauplii as this is a relatively new prey which provided good growth in the present study.

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Appendices

Appendix 1. Outlet mesh size and water exchange rate

Table A1. Outlet mesh size (µm) and water exchange rate (%) in rearing tanks for the different treatments throughout the experimental period (dph).

	Treatments									
	Artemia		**Formulated diet**		**Copepods**		**Cirripedia**		**Cop/Cir**	
Dph	Mesh size (µm)	Water exchange rate (%)	Mesh size (µm)	Water exchange rate (%)	Mesh size (µm)	Water exchange rate (%)	Mesh size (µm)	Water exchange rate (%)	Mesh size (µm)	Water exchange rate (%)
0	750	2400	750	2400	100	300	350	1200	100	300
1	750	2400	750	2400	100	300	350	1200	100	300
2	750	2400	750	2400	100	300	350	1200	100	300
3	750	2400	750	2400	100	300	350	1200	100	300
4	750	2400	750	2400	100	300	350	1200	100	300
5	750	2400	750	2400	100	300	350	1200	100	300
6	750	2400	750	2400	100	300	350	1200	100	300
7	750	2400	750	2400	100	300	350	1200	100	300
8	750	2400	750	2400	100	300	350	1200	100	300
9	750	2400	750	2400	100	300	350	1200	100	300
10	750	2400	750	2400	100	300	350	1200	100	300
11	750	2400	750	2400	100	300	350	1200	100	300
12	750	2400	750	2400	100	300	350	1200	100	300
13	750	2400	750	2400	100	300	350	1200	100	300
14	750	2400	750	2400	100	300	350	1200	100	300
15	750	2400	750	2400	100	600	350	1200	100	600
16	750	2400	750	2400	100	600	350	1200	100	600
17	750	2400	750	2400	750	2400	350	1200	350	1200
18	750	2400	750	2400	750	2400	350	1200	350	1200
19	750	2400	750	2400	750	2400	350	1200	350	1200
20	750	2400	750	2400	750	2400	350	1200	350	1200

Table A1. Outlet mesh size (µm) and water exchange rate (%) in rearing tanks for the different treatments throughout the experimental period (dph).

	Treatments									
	Artemia		**Formulated diet**		**Copepods**		**Cirripedia**		**Cop/Cir**	
Dph	Mesh size (µm)	Water exchange rate (%)	Mesh size (µm)	Water exchange rate (%)	Mesh size (µm)	Water exchange rate (%)	Mesh size (µm)	Water exchange rate (%)	Mesh size (µm)	Water exchange rate (%)
21	750	2400	750	2400	750	2400	350	2400	350	2400
22	750	2400	750	2400	750	2400	350	2400	350	2400
23	750	2400	750	2400	750	2400	350	2400	350	2400
24	750	2400	750	2400	750	2400	350	2400	350	2400
25	750	2400	750	2400	750	2400	350	2400	350	2400
26	750	2400	750	2400	750	2400	750	2400	750	2400
27	750	2400	750	2400	750	2400	750	2400	750	2400
28	750	2400	750	2400	750	2400	750	2400	750	2400
29	750	2400	750	2400	750	2400	750	2400	750	2400
30	750	2400	750	2400	750	2400	750	2400	750	2400
31	750	2400	750	2400	750	2400	750	2400	750	2400
32	750	2400	750	2400	750	2400	750	2400	750	2400
33	750	2400	750	2400	750	2400	750	2400	750	2400
34	750	2400	750	2400	750	2400	750	2400	750	2400
35	750	2400	750	2400	750	2400	750	2400	750	2400

Appendix 2. Feeding amount

Table A2. The amount of *Artemia* (volume/ml), copepod (ind/ml), cirripedia (ind/ml) and formulated diet (g/day) provided to the tanks.

	Treatments									
	Artemia		**FD**	**Copepods**		**Cirripedia**		**Cop/Cir**		
Dph	Art (ml vol)	FD (g/day)	FD (g/day)	Cop (ind/ml)	FD (g/day)	Cir (ind/ml)	FD (g/day)	Cop (ind/ml)	Cir (ind/ml)	FD (g/day)
2	20	-	-	30	-	18	-	30	-	-
3	21	-	4g-1s	30	-	24	-	30	-	-
4	22	-	4g-1s	50	-	30	-	50	-	-
5	23	-	6g-1s	50	-	30	-	50	-	-
6	34	-	6g-1s	50	-	42	-	50	-	-
7	36	-	12g-1s	70	-	42	-	70	-	-
8	38	-	12g-1s	70	-	42	-	70	-	-
9	41	-	24g-2s	90	-	54	-	90	-	-
10	50	-	24g-2s	70	6g-1s	54	-	70	54	-
11	54	-	24g-2s	70	6g-1s	60	-	70	60	-
12	59	-	24g-2s	50	12g-1s	60	-	50	60	-
13	63	-	24g-2s	30	12g-1s	60	-	30	60	-
14	68	-	24g-2s	30	24g-2s	72	-	30	72	-
15	74	-	30g-2s	20	24g-2s	72	-	20	72	-
16	80	-	30g-2s	10	30g-2s	78	-	10	78	-
17	86	-	30g-2s	-	30g-2s	78	-	-	78	-
18	93	-	30g-2s	-	30g-2s	78	-	-	78	-
19	101	-	30g-2s	-	30g-2s	78	-	-	78	-
20	109	-	30g-2s	-	30g-2s	78	-	-	78	-
21	176	6g-1s	30g-2s	-	30g-2s	60	6g-1s	-	60	6g-1s
22	152	6g-1s	30g-2s	-	30g-2s	54	6g-1s	-	54	6g-1s
23	123	12g-1s	30g-2s	-	30g-2s	24	12g-1s	-	24	12g-1s
24	89	12g-1s	30g-2s	-	30g-2s	18	12g-1s	-	18	12g-1s
25	48	24g-2s	30g-2s	-	30g-2s	12	24g-2s	-	12	24g-2s
26		24g-2s	30g-2s	-	30g-2s	-	24g-2s	-	-	24g-2s
27		30g-2s	30g-2s	-	30g-2s	-	30g-2s	-	-	30g-2s
28		30g-2s	30g-2s	-	30g-2s	-	30g-2s	-	-	30g-2s
29		24g-1s	24g-1s	-	24g-1s	-	24g-1s	-	-	24g-1s
30		24g-1s	24g-1s	-	24g-1s	-	24g-1s	-	-	24g-1s
31		24g-1s	24g-1s	-	24g-1s	-	24g-1s	-	-	24g-1s
32		24g-1s	24g-1s	-	24g-1s	-	24g-1s	-	-	24g-1s
33		24g-1s	24g-1s	-	24g-1s	-	24g-1s	-	-	24g-1s
34		24g-1s	24g-1s	-	24g-1s	-	24g-1s	-	-	24g-1s
35		24g-1s	24g-1s	-	24g-1s	-	24g-1s	-	-	24g-1s

Appendix 3. *Artemia* protocol

Working protocol for preparing and enriching *Artemia*. The protocol is made in accordance with producer recommendations and NTNU Sealab facilities.

PROTOCOL FOR Artemia HATCHING, HARVESTING AND ENRICHMENT – STARTRENS 2020

- *Prepare two incubation cones, screw aeration caps, leave valves open, fill them with **60 L of sea water, plug in heaters and start aerations** (Do this daily - the day before the incubation)*
- *Label the cones and note which tanks you use*
- *NB: Prepare the needed Artemia 2 days before they are going to be fed to the fish larvae*

INCUBATING *Artemia* CYSTS

1. Take out the pre-weighed bag containing Artemia-cysts from the fridge, **see to that you take the correct one**
2. Check water temperature in the cone first (25-29°C), make sure the aeration and heater are on. Then pour the cysts into the cone
3. Gently rinse the cysts into the water, they should not stick on the sides
4. It will take about 24h for the hatching to be complete

HARVESTING *Artemia* NAUPLII

1. **Unplug the heater**
2. Close the bottom valve, THEN close the aeration (which is at the top)
3. Let the system settle for about 5 min
4. Prepare the *Artemia* separator: on a nicely levelled surface, put strainers at the outlet
5. **Remove the heater**
6. Unscrew the aeration-cap on the bottom of the cone
7. **Open and close the valve on the tank quickly** to flush out unhatched cysts at the bottom, if there are any
8. Place a bucket underneath and slowly empty the cone water into the bucket, remember to close the valve when the bucket is full. Don't empty the tank too fast, as high pressure/speed will damage/kill the *Artemia*
9. Take the water+*Artemia* and pour gently into the separator to separate hatched nauplii from empty shells/unhatched cysts
10. Take the strainer containing the separated *Artemia* and rinse it with clean seawater into the second cone with clean seawater (60L, 25-29°C, aerated)

11. Continue to empty the cone until it's about 5 cm of water left. This last water you can just flush out (mostly unhatched cysts and are not easily trapped by the magnets)
12. Tip the separator slightly to flush out the last *Artemia* in it and put them in the second cone as well
13. Clean the cone and other equipment (buckets, separator, floor) with warm water and get it ready for use again if needed (60L water, 25-29°C). The aeration-cap at the bottom needs to be rinsed as well before you screw it back on. Make sure the **gasket (round rubber band)** is in place!

1ST ENRICHMENT

1. Take the enrichment (Larviva Multigain) out from the fridge
2. Measure **10g** of the enrichment (Larviva Multigain) in a measuring cup with a spoon
3. Fill ¼ of the measuring cup (with the enrichment in) with luke warm water
4. Stir with a hand-mixer for 3 minutes
5. Remove as much foam as possible with a spoon
6. Rinse the foam which are still around the measuring cup
7. Take it to the other room and empty it into the cone with **the newly hatched *Artemia* nauplii,** rinse the rest with seawater

2ND ENRICHMENT

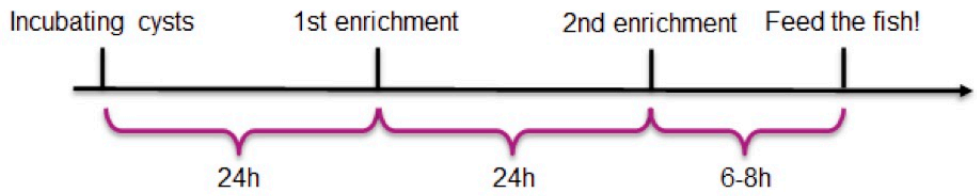
1. See 1st enrichment. Do this early in the morning.

HARVESTING FOR FEEDING THE LARVAE (AFTERNOON AFTER 2ND ENRICHMENT)

1. Prepare two buckets, **first bucket** be marked at a level which indicates for example 5 L
2. Add a small volume of clean seawater (ca 2 L) into the **first bucket** and put it on aeration
3. Add clean sea water to about half in the **second bucket**
4. In the cone with enriched *Artemia,* close the valve at the bottom, the aeration and unplug the heater
5. Wait for about 3 minutes for the heater to cool down, then take it out
6. Put a strainer in the second bucket and slide the bucket under the cone, open the cone to **gently** pour the enriched *Artemia* into the bucket while holding the strainer (the enriched *Atemia* will be soaked in the water but retained in the strainer)
7. Rinse the *Artemia* into the **first bucket**

8. Fill the bucket with clean seawater to the known volume (5 L), increase the aeration to distribute the *Artemia* and take 5 samples of 1 ml each to estimate the density under microscope
9. After estimating the density in for example 5 L **(average density in 1 ml x 5000 ml)**, you will be able to estimate the volume to add in each tank for feeding

TIMELINE



Appendix 4. Producer manual for Cirripedia (Planktonic AS)

Producer manual for thawing, washing and revitalization of Cirripedia nauplii (Planktonic AS).

Procedure for thawing, washing and revitalization of CryoPlankton

Easy method for thawing, washing and revitalization			
Step	To do	Description	Notice
1	Prepare water for revitalization	Decide how much CryoPlankton is needed. Prepare the revitalization water. The water amount should be a minimum of 50 litres per kg CryoPlankton. Fill seawater in the tank and add ice. Use aeration to thaw the ice.	Water temperature < 5°C, salinity 24-36 psu, aeration.
2	Prepare water for thawing	Fill the thawing tank with seawater. The water amount should be a minimum of 25 litres per kg CryoPlankton.	Water temperature 5-12°C (no need for cooling), salinity of 24-36 psu, aeration.
3	Weigh out CryoPlankton	Find a scale, gloves, net, safety glasses and a container to transport the plankton in. Tare the container on the scale, collect the plankton and weigh out the wanted amount. Use safety equipment and protect arms and legs with clothes. Read the material safety data sheet before handling liquid nitrogen.	CryoPlankton that are collected from the thermos should not be transferred back to the thermos. CryoPlankton will not survive the extreme changes in temperature.
4	Thaw CryoPlankton	Add the plankton in the thawing tank with aeration.	The time between taking the plankton out of the thermos until it is transferred to the seawater should not exceed 5 minutes. The vitality of the nauplii could be affected.

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5	Wash CryoPlankton	The plankton can be transferred to the net when the plankton is thawed. Rince with sea water for 5 minutes.	The cryo protection agaent is important to remove because it is toxic for the fish larvae.
6	Revitalize CryoPlankton	Transfer the plankton to the revitalization tank with cold sea water and aeration. CryoPlankton is revitalized after 6 hours and ready to feed to the fish larvae.	

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Appendix 5. Protocol Copepods (*A.tonsa*)

Working protocol for handling, feeding and harvesting of Copepods (*A.tonsa*). The protocol is made in accordance with producer recommendations and NTNU Sealab facilities.

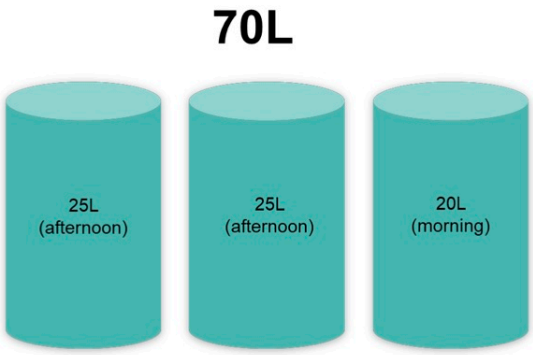
COPEPODS PROTOCOL

*The seawater is warm, to cool it, let it run for a few minutes before using.

AFTERNOON

Harvesting of *Acartia*

1. Check how many litres to filter into each of the 3 buckets (right number for each day will be posted on the wall).
2. Fill all the buckets with some seawater and put aeration before putting the copepods. Filter the right amount into the buckets.
3. When done with filtration, fill up the 3 buckets as show below:



Feeding of *Acartia*

4. Feed the big reservoir with around 20L of microalgae and the "morning" bucket with around 1.5 L.
5. Fill the big reservoir back to 900 L.
6. Around 15 min before feeding to the fish tanks (15:45), feed the "afternoon" buckets with around 2.5 L each. Transport them to CodTech lab with aeration.

MORNING

7. Feed the "morning" bucket with around 2 L of microalgae.
8. Put aeration in the tank to be transported (can use 2 of the microalgae containers with the attached aeration).
9. Transport the bucket to CodTech Lab.
10. Wait around 15 minutes from feeding microalgae, before filling the feeding reservoir to the fish tanks.

Filling the feeding reservoirs

	17h	7h
Normal treatment	8 L/tank	3.3 L/tank
Mix treatment	2.5 L/tank	1.0 L/tank

Appendix 6. Technovit® 7100, dehydration and embedding

Working protocol for dehydration and embedding (Technovit® 7100) obtained from (Marthinsen et al., 2018) with a few adjustments.

Component 1: Basic resin (monomer), 500 mL	2-hydroxyethylmethacrylate (HEMA)
Component 2: Activator (powder), 5 pkg. a 1 g	dibenzoyl peroxide
Component 3: Hardener II (herder), 40 mL	dimethyl sulfoxide

Dehydration fluids:

A: 6 mL distilled water + 2 mL Basic Resin (3 mL/mL) (3:1)

B: 4 mL distilled water + 4 mL Basic Resin (1 mL/mL) (2:2)

Samples were immersed in the first dehydration fluid (A) at 4 °C for 3 hours, before transferred to the second dehydration fluid (B) at room temperature for 2 hours.

Infiltration fluid:

50 ml Basic Resin + 0,5 g Activator (10 mg/mL)

A magnetic stirrer was used until the powder was completely dissolved. The dehydrated samples were immersed in the infiltration fluid and placed on a digital shaker (ROCKER 3D digital, IKA®, USA) overnight (12 hours or more). The infiltration fluid was stored at 4 °C for a maximum of four weeks.

Polymerization fluid:

1.5 mL infiltration fluid + 100 uL Hardener II (0.15 mL/mL)

The two components were mixed for 3-5 minutes and used immediately, as the polymerization process was very rapid. The samples were placed and oriented in individual embedment molds, which were partly filled with the polymerization fluid. An adapter (holding the block steady when attached to the microtome) was then placed over the mold containing the sample, and the mold was filled with polymer through a hole in the adapter. The embedment was completed after 40- 120 minutes at room temperature.

Appendix 7. Mean dry weight per tank

Table A3. Mean dry weight in *C.lumpus* from 2-34 dph in each tank with corresponding standard error (± SE). *N* is the total number of larvae.

Dph	Group	Tank	Mean DW ± SE (mg/larva)	N	Dph	Group	Tank	Mean DW ± SE (mg/larva)	*N*
2	All	-	0.91 ± 0.05	15					
9	Art	1	1.04 ± 0.06	5	**29**	Art	1	2.92 ± 0.25	15
		7	1.17 ± 0.10	5			7	3.89 ± 0.20	15
		15	0.98 ± 0.04	5			15	3.66 ± 0.21	15
	FD	2	0.90 ± 0.06	5		FD	2	2.27 ± 0.21	15
		10	0.98 ± 0.03	5			10	2.42 ± 0.16	15
		12	0.82 ± 0.07	5			12	2.57 ± 0.11	15
	Cop/ FD	6	0.92 ± 0.05	5		Cop/ FD	6	2.27 ± 0.17	15
		11	1.02 ± 0.04	5			11	2.10 ± 0.13	15
		13	0.93 ± 0.11	5			13	2.18 ± 0.19	15
	Cir	4	0.80 ± 0.02	5		Cir	4	2.97 ± 0.24	15
		8	0.73 ± 0.11	5			8	3.11 ± 0.23	15
		14	1.01 ± 0.07	5			14	2.97 ± 0.21	15
	Cop/ Cir	3	1.05 ± 0.08	5		Cop/ Cir	3	2.12 ± 0.28	15
		5	1.04 ± 0.07	5			5	2.19 ± 0.15	15
		9	0.86 ± 0.06	5			9	2.60 ± 0.17	15
15	Art	1	1.73 ± 0.13	5	**34**	Art	1	4.35 ± 0.24	15
		7	1.68 ± 0.13	5			7	4.11 ± 0.17	15
		15	1.93 ± 0.09	5			15	4.82 ± 0.25	15
	FD	2	0.83 ± 0.06	5		FD	2	3.54 ± 0.23	15
		10	0.90 ± 0.09	5			10	2.96 ± 0.19	15
		12	1.16 ± 0.12	5			12	3.53 ± 0.17	15
	Cop/ FD	6	1.07 ± 0.09	5		Cop/ FD	6	2.85 ± 0.23	15
		11	1.14 ± 0.08	5			11	2.96 ± 0.24	15
		13	1.09 ± 0.09	5			13	3.23 ± 0.21	15
	Cir	4	1.17 ± 0.08	5		Cir	4	4.70 ± 0.26	15
		8	1.16 ± 0.09	5			8	4.38 ± 0.38	15
		14	1.29 ± 0.08	6			14	4.12 ± 0.38	15
	Cop/ Cir	3	1.13 ± 0.07	5		Cop/ Cir	3	3.86 ± 0.27	15
		5	1.24 ± 0.05	5			5	3.12 ± 0.29	15
		9	1.13 ± 0.06	5			9	3.16 ± 0.30	15
21	Art	1	3.03 ± 0.12	10					
		7	2.59 ± 0.12	10					
		15	2.59 ± 0.09	10					
	FD	2	1.36 ± 0.09	10					
		10	1.41 ± 0.07	10					
		12	1.65 ± 0.11	10					
	Cop/ FD	6	0.93 ± 0.05	10					
		11	1.38 ± 0.09	10					
		13	1.18 ± 0.14	10					
	Cir	4	1.90 ± 0.12	10					
		8	1.90 ± 0.15	10					
		14	1.73 ± 0.09	10					
	Cop/ Cir	3	1.96 ± 0.14	10					
		5	1.90 ± 0.07	10					
		9	1.51 ± 0.05	10					

Appendix 8. Daily weight increase (DWI)

Table A4. Daily weight increase in *C.lumpus* on different dph interval in each tank.

Dph interval	Group	Tank	DWI (%/day)	Dph interval	Group	Tank	DWI (&/day)
2-9	Art	1	1.97	**21-34**	Art	1	2.82
		7	3.73			7	3.63
		15	1.10			15	4.87
	FD	2	-0.10		FD	2	7.60
		10	1.12			10	5.87
		12	-1.40			12	6.05
	Cop/ FD	6	0.27		Cop/ FD	6	9.03
		11	1.66			11	6.06
		13	0.34			13	8.07
	Cir	4	-1.75		Cir	4	7.23
		8	-3.02			8	6.62
		14	1.59			14	6.90
	Cop/ Cir	3	2.10		Cop/ Cir	3	5.34
		5	2.05			5	3.87
		9	-0.65			9	5.86
9-21	Art	1	9.35	**2-34**	Art	1	5.03
		7	6.85			7	4.85
		15	8.48			15	5.36
	FD	2	3.54		FD	2	4.35
		10	3.10			10	3.94
		12	5.97			12	4.35
	Cop/ FD	6	0.04		Cop/ FD	6	3.65
		11	2.56			11	3.77
		13	2.01			13	4.05
	Cir	4	7.47		Cir	4	5.28
		8	8.31			8	5.05
		14	4.58			14	4.85
	Cop/ Cir	3	5.37		Cop/ Cir	3	4.64
		5	5.14			5	3.94
		9	4.75			9	3.99

Appendix 9. Mean standard length per tank

Table A5. Mean standard length in *C.lumpus* from 2-34 dph in each tank with corresponding standard error (± SE). *N* is the total number of larvae.

Dph	Group	Tank	Mean DW ± SE (mg/larva)	N	Dph	Group	Tank	Mean DW ± SE (mg/larva)	*N*
2	All	-	5.94 ± 0.10	15					
9	Art	1	6.39 ± 0.26	5	**29**	Art	1	8.51 ± 0.20	15
		7	6.78 ± 0.16	5			7	9.29 ± 0.13	15
		15	6.62 ± 0.07	5			15	9.04 ± 0.16	15
	FD	2	6.49 ± 0.11	5		FD	2	7.70 ± 0.26	15
		10	6.61 ± 0.07	5			10	8.01 ± 0.16	15
		12	6.34 ± 0.13	5			12	8.13 ± 0.13	15
	Cop/ FD	6	6.65 ± 0.14	5		Cop/ FD	6	7.80 ± 0.18	15
		11	6.44 ± 0.08	5			11	7.66 ± 0.15	15
		13	6.61 ± 0.15	5			13	7.71 ± 0.19	15
	Cir	4	6.60 ± 0.05	5		Cir	4	8.54 ± 0.20	15
		8	6.25 ± 0.21	5			8	8.51 ± 0.19	15
		14	6.46 ± 0.12	5			14	8.43 ± 0.19	15
	Cop/ Cir	3	6.61 ± 0.10	5		Cop/ Cir	3	7.62 ± 0.27	15
		5	6.40 ± 0.15	5			5	7.70 ± 0.15	15
		9	6.50 ± 0.08	5			9	8.08 ± 0.15	15
15	Art	1	7.87 ± 0.13	5	**34**	Art	1	9.26 ± 0.13	15
		7	7.51 ± 0.11	5			7	9.19 ± 0.11	15
		15	7.79 ± 0.07	5			15	9.54 ± 0.15	15
	FD	2	6.59 ± 0.16	5		FD	2	8.57 ± 0.17	15
		10	6.77 ± 0.12	5			10	8.23 ± 0.17	15
		12	7.05 ± 0.15	5			12	8.76 ± 0.14	15
	Cop/ FD	6	7.04 ± 0.04	5		Cop/ FD	6	8.09 ± 0.18	15
		11	7.04 ± 0.16	5			11	8.28 ± 0.19	15
		13	7.14 ± 0.12	5			13	8.46 ± 0.15	15
	Cir	4	7.08 ± 0.08	5		Cir	4	9.25 ± 0.15	15
		8	6.80 ± 0.39	5			8	9.14 ± 0.25	15
		14	7.37 ± 0.15	6			14	9.04 ± 0.26	15
	Cop/ Cir	3	7.24 ± 0.19	5		Cop/ Cir	3	8.78 ± 0.19	15
		5	7.40 ± 0.14	5			5	8.22 ± 0.21	15
		9	7.16 ± 0.07	5			9	8.29 ± 0.24	15
21	Art	1	8.62 ± 0.08	10					
		7	8.12 ± 0.11	10					
		15	8.28 ± 0.09	10					
	FD	2	7.37 ± 0.11	10					
		10	7.41 ± 0.10	10					
		12	7.46 ± 0.09	10					
	Cop/ FD	6	6.94 ± 0.07	10					
		11	7.27 ± 0.11	10					
		13	7.24 ± 0.15	10					
	Cir	4	7.79 ± 0.16	10					
		8	7.80 ± 0.15	10					
		14	7.68 ± 0.10	10					
	Cop/ Cir	3	7.85 ± 0.08	10					
		5	7.91 ± 0.08	10					
		9	7.57 ± 0.07	10					

Appendix 10. Mean standard length of 250 larvae per tank

Table A6. Mean standard length in *C.lumpus* on 35 dph in each tank with corresponding standard error (± SE). *N* is the total number of larvae.

Dph	Group	Tank	Mean DW ± SE (mg/larva)	N
35	Art	1 7 15	9.29 ± 0.04 9.25 ± 0.04 9.34 ± 0.04	250 250 250
	FD	2 10 12	8.67 ± 0.04 8.39 ± 0.04 8.55 ± 0.03	250 250 250
	Cop/FD	6 11 13	8.17 ± 0.04 8.54 ± 0.04 8.42 ± 0.04	250 250 250
	Cir	4 8 14	9.05 ± 0.04 8.79 ± 0.05 8.95 ± 0.04	250 250 250
	Cop/Cir	3 5 9	8.70 ± 0.05 8.34 ± 0.05 8.46 ± 0.04	250 250 250

Appendix 11. Number of lumpfish larvae per tank

Table A7. Estimated number of lumpfish larvae alive in each tank from 2-35 dph. The estimation is based on the remaining larvae left at 35 dph, sampled larvae and registered dead larvae.

	Treatments								
	Artemia			**FD**			**Cop/FD**		
	Tank								
Dph	**1**	**7**	**15**	**2**	**10**	**12**	**6**	**11**	**13**
2	6663	7017	4361	9314	5923	5680	6044	6982	3570
3	6610	7000	4361	9276	5897	5655	5993	6982	3570
4	6610	7000	4341	9267	5875	5544	5993	6831	3545
5	6594	6999	4341	9264	5863	5538	5911	6805	3483
6	6591	6995	4338	9258	5859	5524	5876	6777	3432
7	6590	6992	4335	9251	5851	5519	5863	6740	3411
8	6586	6990	4332	9249	5850	5517	5854	6732	3409
9	6572	6971	4330	9230	5848	5515	5813	6709	3378
10	6567	6971	4324	9224	5848	5512	5788	6686	3355
11	6565	6963	4324	9217	5842	5510	5781	6681	3353
12	6565	6958	4323	9209	5832	5509	5776	6678	3350
13	6564	6958	4323	9204	5819	5506	5768	6648	3347
14	6562	6953	4321	9199	5804	5489	5761	6630	3347
15	6562	6947	4321	9192	5793	5476	5752	6601	3347
16	6559	6940	4317	9165	5732	5459	5737	6537	3325
17	6557	6933	4312	9149	5732	5442	5728	6506	3294
18	6554	6927	4306	9109	5715	5423	5722	6498	3286
19	6552	6925	4304	9091	5701	5421	5692	6486	3273
20	6550	6923	4296	9082	5662	5409	5659	6466	3264
21	6528	6920	4290	9073	5632	5389	5606	6448	3260
22	6520	6920	4282	9066	5596	5370	5590	6427	3254
23	6516	6909	4278	9057	5543	5353	5569	6391	3232
24	6493	6888	4263	9047	5531	5352	5520	6364	3219
25	6465	6861	4243	9038	5514	5351	5473	6317	3204
26	6434	6831	4231	9030	5463	5326	5403	6268	3179
27	6401	6819	4215	9026	5424	5323	5347	6224	3161
28	6389	6791	4207	9019	5386	5313	5291	6182	3126
29	6383	6778	4204	9013	5295	5310	5231	6121	3103
30	6378	6764	4196	9004	5236	5299	5176	6112	3090
31	6378	6748	4193	8996	5184	5298	5156	6092	3080
32	6363	6725	4183	8983	5115	5295	5121	6080	3071
33	6357	6705	4178	8967	5079	5295	5103	6078	3068
34	6351	6695	4172	8961	5046	5295	5079	6075	3065
35	6347	6686	4171	8958	5028	5292	5070	6070	3064

Table A7. Estimated number of lumpfish larvae alive in each tank from 2-35 dph. The estimation is based on the remaining larvae left at 35 dph, sampled larvae and registered dead larvae.

	Treatments					
	Cir			**Cop/Cir**		
	Tank					
Dph	**4**	**8**	**14**	**3**	**5**	**9**
2	6359	6220	6774	8536	11424	8892
3	6114	6182	6774	8438	11412	8881
4	6114	6178	6503	8438	11412	8881
5	6092	6175	6380	8391	11406	8869
6	6083	6174	6297	8381	11396	8865
7	6080	6171	6282	8378	11394	8844
8	6077	6169	6261	8369	11392	8828
9	6067	6165	6255	8357	11380	8776
10	6063	6154	6243	8355	11373	8759
11	6061	6150	6228	8351	11365	8743
12	6061	6142	6217	8350	11351	8736
13	6056	6140	6217	8256	11293	8719
14	6054	6138	6216	8240	11243	8711
15	6050	6135	6215	8222	11200	8704
16	6040	6133	6214	8193	11174	8678
17	6035	6132	6212	8190	11152	8667
18	6030	6128	6207	8181	11124	8648
19	6028	6128	6207	8173	11120	8633
20	6023	6126	6205	8169	11097	8610
21	6018	6118	6203	8165	11076	8594
22	5988	6099	6189	8155	11076	8544
23	5983	6093	6184	8144	11029	8532
24	5981	6087	6176	8140	10988	8526
25	5973	6078	6169	8112	10950	8485
26	5969	6057	6150	8090	10935	8435
27	5963	6048	6134	8073	10883	8327
28	5954	6011	6127	8049	10848	8213
29	5948	5992	6122	8042	10812	8141
30	5934	5977	6108	8019	10754	8092
31	5930	5973	6100	8011	10701	8072
32	5925	5968	6096	8000	10660	8027
33	5912	5945	6086	7984	10615	8002
34	5908	5942	6080	7963	10586	7996
35	5904	5937	6075	7948	10568	7978

Appendix 12. Number of red fibers in individual lumpfish larvae

Table A8. Number of red fibers in individual larvae of *C.lumpus* from 2-35 dph.

Dph	Group	Number of red fibers (n)	Dph	Group	Number of red fibers (n)	Dph	Group	Number of red fibers (n)
2	**All**	33 43 40 42 37						
9	**Art**	54 49 48 46 40	**21**	**Art**	43 39 32 51 41	**35**	**Art**	89 111 115 110 127
	FD	31 38 39 35 46		**FD**	42 34 37 50 39		**FD**	69 77 76 69 75
	Cop/FD	48 38 42 41 41		**Cop/FD**	36 43 37 37 46		**Cop/FD**	53 67 59 57 58
	Cir	44 36 38 69 42		**Cir**	54 43 41 53 33		**Cir**	117 71 65 70 56
	Cop/Cir	38 45 35 44 37		**Cop/Cir**	36 34 48 36 45		**Cop/Cir**	102 102 117 104 86

Appendix 13. Mean fiber size of 30 largest red fibers in individual lumpfish larvae

Table A9. Mean number of 30 largest red fibers in individual larvae of *C.lumpus* from 2-35 dph.

Dph	Group	Mean fiber size of 30 largest red fibers (μm^2)	Dph	Group	Mean fiber size of 30 largest red fibers (μm^2)	Dph	Group	Mean fiber size of 30 largest red fibers (μm^2)
2	**All**	121 145 123 132 132						
9	**Art**	123 116 134 118 126	**21**	**Art**	164 114 140 173 212	**35**	**Art**	203 267 129 186 210
	FD	121 102 133 122 121		**FD**	69 83 155 82 88		**FD**	186 132 173 162 190
	Cop/FD	128 91 99 99 102		**Cop/FD**	66 104 101 67 103		**Cop/FD**	118 100 164 127 157
	Cir	118 122 110 86 88		**Cir**	105 148 180 206 111		**Cir**	227 134 224 174 139
	Cop/Cir	106 105 98 92 91		**Cop/Cir**	104 154 165 111 116		**Cop/Cir**	285 241 143 224 137

Appendix 14. Total red muscle area in individual lumpfish larvae

Table A10. Total area of red muscle in individual larvae of *C.lumpus* from 2-35 dph.

Dph	Group	Total red area (µm²)	Dph	Group	Total red area (µm²)	Dph	Group	Total red area (µm²)
2	**All**	3729 4745 4264 4660 4166						
9	**Art**	4449 4142 4853 4466 4336	**21**	**Art**	5755 3706 4208 6345 6926	**35**	**Art**	9547 12 483 6463 8941 9902
	FD	3632 3432 4258 3832 4403		**FD**	2244 2569 4844 2877 2906		**FD**	7955 5282 6958 6516 7965
	Cop/ FD	4795 2972 3290 3426 3456		**Cop/ FD**	2022 3380 3136 2093 3457		**Cop/ FD**	4657 4173 5745 4686 6056
	Cir	4139 3831 3668 3715 3015		**Cir**	3563 4771 5792 7164 3340		**Cir**	13 192 5891 9142 7765 5242
	Cop/ Cir	3516 3747 3044 3338 2866		**Cop/ Cir**	3214 4706 5933 3509 3831		**Cop/ Cir**	14 010 10 758 6902 9304 5523

Appendix 15. Number of white fibers in individual lumpfish larvae

Table A11. Number of white fibers in individual larvae of *C.lumpus* from 2-35 dph.

Dph	Group	Number of white fibers (n)	Dph	Group	Number of white fibers (n)	Dph	Group	Number of white fibers (n)
2	**All**	519 533 615 518 358						
9	**Art**	621 545 666 577 526	**21**	**Art**	776 671 659 839 776	**35**	**Art**	847 840 898 780 849
	FD	596 490 474 440 619		**FD**	570 514 677 643 618		**FD**	851 630 721 673 823
	Cop/FD	657 660 552 680 568		**Cop/FD**	514 655 439 573 661		**Cop/FD**	732 761 785 728 748
	Cir	445 519 641 878 595		**Cir**	681 736 743 788 763		**Cir**	838 779 869 787 743
	Cop/Cir	585 538 580 622 660		**Cop/Cir**	551 742 675 530 661		**Cop/Cir**	840 820 775 783 741

Appendix 16. Mean fiber size of 350 largest white fibers in individual lumpfish larvae

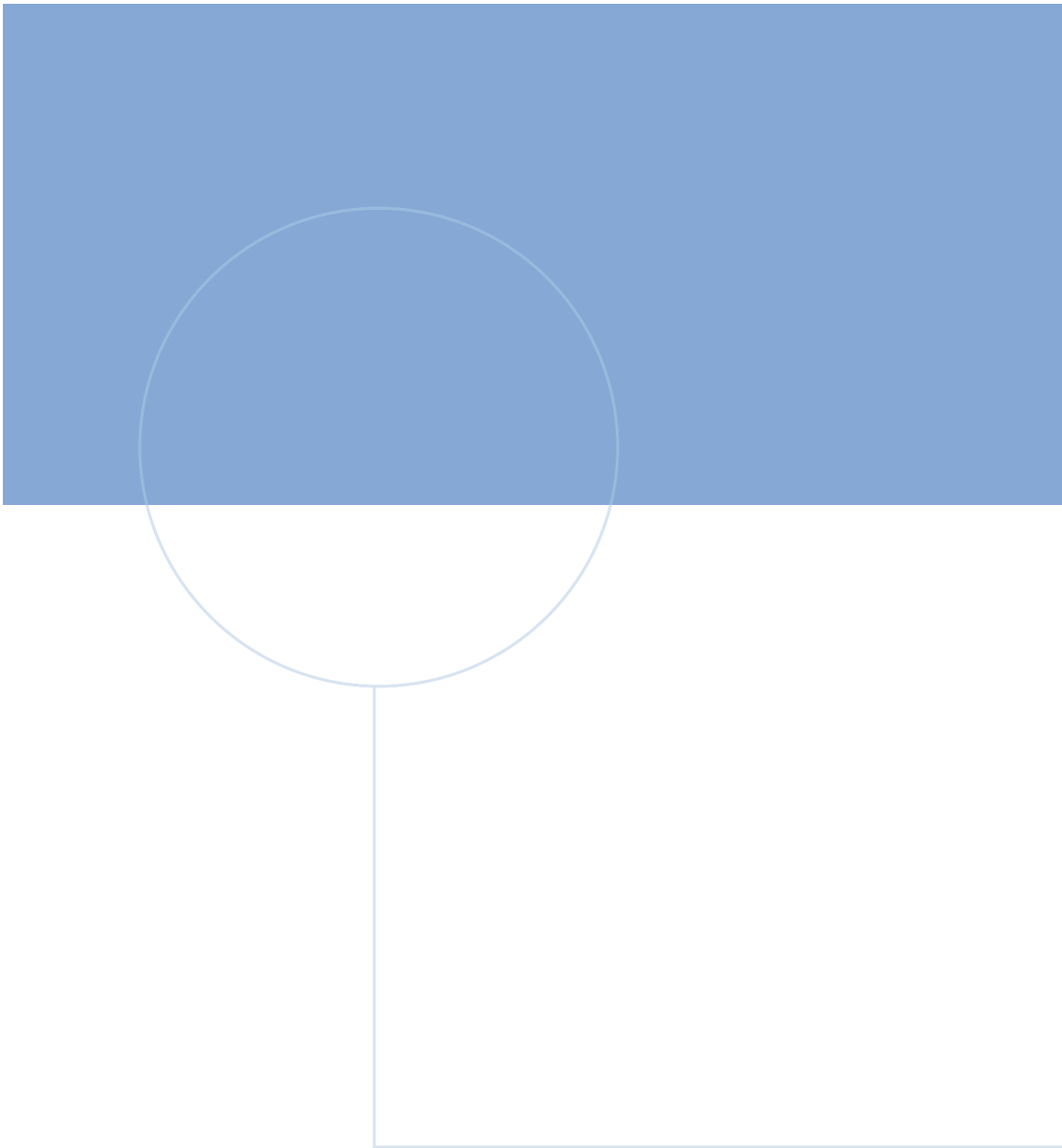
Table A12. Mean number of 350 largest white fibers in individual larvae of *C.lumpus* from 2-35 dph.

Dph	Group	Average cell size of 350 largest white fibers (μm^2)	Dph	Group	Average cell size of 350 largest white fibers (μm^2)	Dph	Group	Average cell size of 350 largest white fibers (μm^2)
2	**All**	97 126 107 101 104						
9	**Art**	141 117 149 159 102	**21**	**Art**	405 336 344 415 360	**35**	**Art**	527 616 613 517 668
	FD	107 104 156 116 145		**FD**	119 163 263 250 136		**FD**	562 319 392 401 491
	Cop/ FD	167 115 128 158 151		**Cop/ FD**	129 285 259 210 222		**Cop/ FD**	229 379 393 364 386
	Cir	150 123 165 80 143		**Cir**	308 346 420 434 335		**Cir**	712 505 721 528 444
	Cop/ Cir	122 118 137 140 162		**Cop/ Cir**	282 315 278 243 283		**Cop/C ir**	701 602 511 710 533

Appendix 17. Total white muscle area of in individual lumpfish larvae

Table A13. Total area of white muscle in individual larvae of *C.lumpus* from 2-35 dph.

Dph	Group	Total white area (µm²)	Dph	Group	Total white area (µm²)	Dph	Group	Total white area (µm²)
2	**All**	38 133 49 401 46 358 39 691 36 335						
9	**Art**	58 901 47 366 67 275 65 170 41 210	**21**	**Art**	189 485 149 083 151 759 211 935 174 589	**35**	**Art**	256 266 285 556 297 908 241 888 311 955
	FD	45 886 40 565 58 629 42 882 62 963		**FD**	50 213 64 053 114 094 101 900 57 599		**FD**	277 873 136 341 174 262 168 050 228 160
	Cop/ FD	73 638 50 151 52 773 70 532 63 317		**Cop/ FD**	49 499 121 143 93 204 86 104 97 502		**Cop/ FD**	106 032 169 126 178 676 164 269 178 400
	Cir	55 537 49 622 71 041 39 704 58 223		**Cir**	136 059 160 183 190 825 207 729 161 184		**Cir**	353 812 236 129 338 177 240 235 199 784
	Cop/ Cir	51 686 47 753 56 833 60 673 72 059		**Cop/ Cir**	106 387 139 135 119 940 92 635 121 782		**Cop/ Cir**	354 055 288 484 231 336 320 215 231 836



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