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Timing and selectivity of mortality in reared Atlantic cod revealed by otolith analysis

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Abstract In juvenile fish production, large samples of known-aged material can be sampled at pre-determined time intervals from the same population. This enables an accurate determination of size-selective mortality by means of repeated samplings of fish and comparison of otolith size-at-age based on samples from different dates. An example is provided from an experiment with larval and juvenile Atlantic cod, *Gadus morhua*, where groups fed smaller sized enriched rotifers did not reveal any size-selective mortality during weaning to formulated feed, while those groups that were fed larger live natural zooplankton lost a significant fraction of the smaller-sized individuals during the same time period. This was contrary to the overall mortality which was higher among the rotifer fed groups during weaning. Part of this difference may be attributed to size differences between the groups, where larger zooplankton fed larvae were more prone to engage in cannibalistic behaviour.

Keywords Selective mortality · Aquaculture · Juvenile fish production · *Gadus morhua* · Sampling · Otolith microstructure

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Introduction

Otolith microstructure analysis has for some decades been used to infer ages, growth rates and selective mortality among field caught fish larvae (e.g., Campana and Hurley 1989; Suthers and Sundby 1993; Meekan and Fortier 1996). Age and growth information from otolith analysis may seem superfluous in aquaculture fish production where the age of the offspring is already typically known and population growth can be derived from easily accessible size-at-age data. However, the use of repeated samplings within the same population readily facilitates the estimation of size-selective mortality during important life history transitions like the onset of external feeding, metamorphosis or weaning onto formulated feeds (Mosegaard et al. 2002). Furthermore, since in a culture setting the age at sampling is typically known, otolith sizes at previous ages can readily be extracted as long as the outer increments are clear and validated to be daily (e.g., Folkvord et al. 2004). Problems relating to the timing of initial increment formation and increment formation rate during the first days of larval life (e.g., Fox et al. 2003), can thus be avoided.

Here we provide an example using otolith microstructure analysis from an aquaculture rearing experiment with larval cod, *Gadus morhua*, where the mortality pattern during the period of weaning onto formulated feed was the focus of our study. The larvae had previously been subject to two different feed types, enriched rotifers (R) and live natural zooplankton (Z), at two nominal initial larval densities, Low (L) and

High (H). It has previously been shown that weaning in cod can result in agonistic behaviour and cannibalism due to an induced food restriction caused by the reduced availability of the preferred live prey during the transition to formulated feeds (Folkvord 1991; Baras and Jobling 2002). The initiation of agonistic behaviours in cod larvae have also been documented to occur earlier during ontogeny than previously thought (Puvanendran et al. 2008), but documentation of the timing and extent of the selective mortality has proven difficult to assess. We therefore chose to use otolith microstructure analysis as a means to document the magnitude and timing of selective mortality in larval cod, and we carried out repeated samplings around the weaning period when the larvae change their diet from live feed to formulated feed. In this case it was expected that the previous feeding experiences and prey preferences would influence the readiness to switch to formulated feed (Koedijk et al. 2010). Furthermore, we also expected the extent of size-selective mortality to be higher in the groups exhibiting the longest transition to formulated feed due to elevated hunger levels, intracohort competition and cannibalism.

Materials and methods

Cod larvae were made available from an aquaculture experiment investigating the combined effects of larval rearing density and feed type (Koedijk et al. 2010). Larvae were reared in replicate tanks at two nominal initial densities (14 (L) and 82 (H) larvae L^{-1}), and fed with either enriched rotifers (R) from a commercial juvenile cod producer, or natural live zooplankton (Z). The tank size was 1×1 m (0.6×0.6 m for ZH group), with a rearing volume of 200 L (130 L for ZH group). The temperature in the tanks increased from around 8°C at the beginning to about 9°C towards the end of the experiment. The larvae were fed their respective prey from 1 day post hatch (dph) (April 10) until 36 dph (May 15) when co-feeding with a formulated feed (EWOS AgloNorse Standard) was initiated. The densities of prey were adjusted daily and increased during the live feed period from 2 to 3 ml^{-1} (L groups) and 5 to 8 ml^{-1} (H groups) to accommodate for differences in grazing pressure. The natural zooplankton consisted mainly of various stages of calanoid copepods (*Temora sp.*). During weaning the amount of live prey was gradually reduced and from 43 dph onwards only formulated feed was offered to the fish. Larvae from a total of eight tanks were sampled on a weekly basis and used in this study. All remaining larvae in the tanks were recounted on 36 dph and at termination of the experiment (50 dph) to provide accurate survival estimates during the experiment, except in the RH tanks on 36 dph where the number of larvae was estimated by volumetric sub-sampling. For further details of rearing procedures see Koedijk et al. (2010).

Otoliths (lapilli) were removed from 149 larvae sampled on 36 dph (May 15), 43 dph (May 22) and 50 dph (May 29) to assess size-selective mortality within each group during weaning (Table 1). Otoliths were mounted in thermoplastic glue on a glass slide using micro needles and polished if necessary to reveal daily increments. Otolith radii of lapilli were measured individually under 400X using an Olympus BX 61 microscope and ImageJ ® software. The radius was measured from the centre of the core to the edge, perpendicular to the widest part of the otolith (Fig. 1). Estimation of previous otolith size at given sampling date (May 15 and May 22) was performed by measuring otolith radius along the same transect as for the outer radius but subtracting the distance

Table 1 Number of otolith samples, average group specific survival during weaning and overall survival of respective groups during the experiment

Group	Otolith samples (36, 43, 50 dph)	Weaning survival (36–50 dph)	Overall survival (1–50 dph)
Rotifer Low (RL)	10, 8, 20	41.2% ^{ab}	26.4% ^{a}
Rotifer High (RH)	10, 10, 20	15.2% ^{b}	10.9% ^{b}
Zooplankton Low (ZL)	8, 10, 18	56.8% ^{a}	25.9% ^{a}
Zooplankton High (ZH)	9, 9, 17	46.4% ^{ab}	8.9% ^{b}

Different letters following values indicate significantly different group means ($p<0.05$, two-way ANOVA)

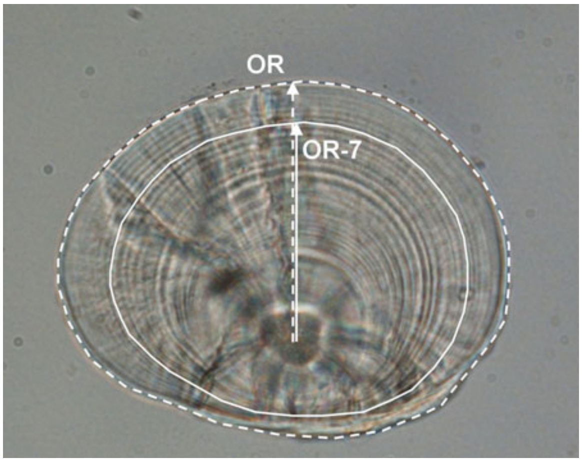

Fig. 1 Lapillus otolith of cod from the RL group sampled on 43 dph. OR and *dashed line arrow* reflects otolith radius-at-date of sampling (May 22) and OR-7 and *solid line* reflects otolith radius 7 days prior to sampling (May 15). *Arrows* are shifted for clarity (OR=64 µm)

corresponding to 7 and 14 daily increments from the outer edge on samples taken at 43 dph (May 22) and 50 dph (May 29) (Fig. 1). These back-calculated otolith radii-at-date were then compared to the outer otolith radii of each specific date to estimate size-selective mortality within each group.

Statistical differences in mean fish and otolith size-at-age between groups was analysed by a three-way ANOVA with feed type, larval density as independent fixed factors and tank as a random factor nested in feed type and density. For the estimation of size selective mortality based on otolith size-at-age, we used a four-way ANOVA with age of sampling, feed type, larval density as independent fixed factors and as a random factor nested in feed type and density. In both cases, highest order non-significant interactions were removed from the models. Group specific survival was assessed using values from replicate tanks in a factorial two-way ANOVA with feed type and larval density as factors. In case of more than two treatment levels, Tukey HSD post-hoc tests were used to infer differences between groups. All test results were considered significant at a level of 0.05. Although the means of the otolith sizes at age within each replicate were adequately estimated to detect group differences, too few larvae were available to generate precise cumulative size distributions on a per tank basis (Folkvord et al. 2009). All larvae from the main food treatment groups were therefore combined on a per date basis to contrast the overall distribution patterns of Z and R groups prior to and during weaning.

Results

Larval cod fed live zooplankton (Z-groups) eventually grew faster than those fed enriched rotifers and averaged (±SD) 12.1 (2.2) mm at 36 dph, compared to 9.7 (1.0) to 7.6 (0.8) mm in the RL- and RH-groups (Fig. 2). At the end of the experiment on 50 dph the average larval sizes were 18.8 (2.7), 17.6 (2.2), 12.1 (1.7) and 9.2 (1.2) mm in the ZL, ZH, RL and RH groups respectively (three-way nested ANOVA, $p<0.001$, Koedijk et al. 2010). The relative size difference was similar between groups as estimated by coefficient of variation (CV) of length which ranged from 12.5 to 14. Differences in otolith mean sizes-at-age were also evident where the Z-group larvae had larger otoliths than the R-group larvae from 36 dph (three-way nested ANOVAs, $p<0.05$). A density effect was also apparent at 50 dph, mainly due to a lower growth of the RH group (three-way nested ANOVAs, $p<0.05$).

Survival was higher among the Z-groups and in the low density groups during the weaning period (36–50 dph, two-way ANOVA, $p<0.05$, Table 1), although no clear patterns in size-selective mortality could be induced from these figures. Over the whole experimental

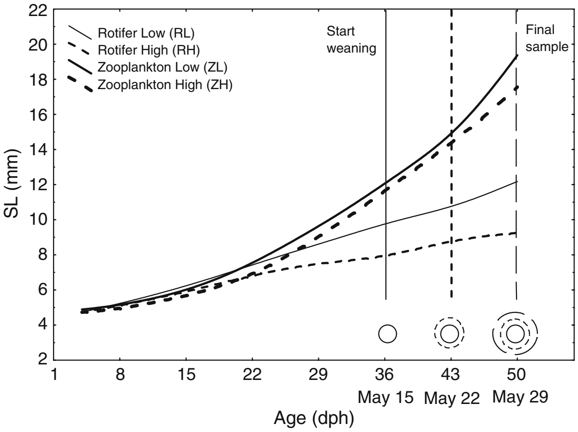

Fig. 2 Standard length-at-age (lowess fit) of groups of cod larvae in low (L, *solid lines*) and high (H, *dashed lines*) larval density groups, fed either rotifers (R, *thin lines*) or natural zooplankton (Z, *thick lines*). *Vertical lines* indicate time of otolith sampling and start of weaning (*solid line* for May 15, *dashed line* for May 22, and long *dashed line* for May 29). *Circles* on lower right signify otolith sizes at respective sampling periods

period (1–50 dph), no differences in survival between the larval diet groups were observed, but those raised at a lower larval density had a significantly higher survival than those from the higher larval density (approx. 25 vs 10%, $p<0.01$, two-way ANOVA, Table 1).

The otolith radius-at-age of R-group larvae sampled on May 15 (36 dph) did not differ from back-calculated otolith sizes to this date from larvae sampled on May 22 or May 29 (Fig. 3, Tukey HSD test, $p>0.05$). In the Z-groups, otoliths radii on May 15 and the back-calculated May 15 radii from fish sampled on May 22 did not differ significantly either. However, unlike for the R-group, the back-calculated radii of otoliths from the Z-group sampled on May 29 had significantly larger sizes than those from fish sampled on May 15 and May 22 (Fig. 3, four-way nested ANOVA, Age*Feed interaction, $p<0.0001$, Table 2), clearly indicating size-selective mortality of the smallest larvae had taken place between May 22 and May 29.

A similar pattern was observed when analysing the otolith size-at-age from larvae sampled on May 22 (43 dph), where only Z-group larvae sampled on May 29 were larger than their corresponding group sampled on May 22 (Fig. 3, four-way nested ANOVA, Age*Feed interaction, $p<0.0001$, Table 2). Overall there were no significant differences in otolith radius of larvae from different replicate tanks (four-way ANOVAs, $0.09<p<0.12$, Table 2). As in the size-at-age analysis above, there were indications that the difference between the RL-group and RH-group was larger than that of the ZL- and ZH-groups (four-way nested ANOVAs, Feed*Density interactions, $0.06<p<0.09$, Table 2).

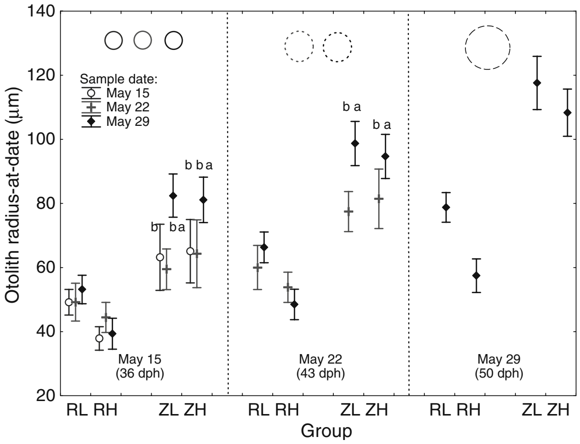

Fig. 3 Otolith sizes from groups of cod larvae sampled at different dates and corresponding ages. Different letters at respective groups indicate significantly different otolith sizes-at-date of larvae sampled at different dates ($p<0.05$, Tukey HSD test). R and Z represent rotifer and zooplankton groups, and L and H low and high density groups respectively. *Circles* on top represent number of samples from different dates available for each group at each date. Data from replicate tanks are combined for visual clarity

Among the R-group larvae only minor differences in the combined cumulative size distributions are apparent, with slightly more extreme sizes observed in the May 29 sample, possibly due to a larger sample size (Table 1, Fig. 4). A marked difference in cumulative size distributions at 43 dph can be seen between the Z-group larvae sampled on May 22 and those sampled one week later. About 50% of the fish from the May 22 sample had otoliths smaller than 75 µm, while this was reduced to less than 10% in the May 29 sample (Fig. 4). Given that not much more than 40% of the fish died or disappeared during this period, it can be concluded that nearly all of the mortality in this group was size selective.

Discussion

Documentation of size-selective mortality by use of otolith microstructure analysis has widely been used in field investigations (e.g., Meekan and Fortier 1996; Nielsen and Munk 2004; Shoji and Tanaka 2006), but this study represents one of the first attempts to use this method in an aquaculture setting. Differences in survival between density groups have previously been observed in cultured fish larvae and have been attributed to increased food competition and aggression (Baras and Jobling 2002), while in other experiments with cod, walleye (*Stizostedion vitreum*) and weakfish (*Cynoscion regalis*), only growth and not survival was reduced as initial larval density was increased (Fox and Flowers 1990; Duffy and Epifanio 1994; Baskerville-Bridges and Kling 2000b). The timing and underlying causes of the mortality may explain these patterns, but in a large scale juvenile fish production setting the actual population size is normally only sporadically assessed. Otolith microstructure analysis has the potential to provide useful information about the magnitude and timing of mortality during juvenile fish production.

A higher selective mortality in the Z-groups may be due to the larger absolute size and size differences

Table 2 Results of four-way nested ANOVAs with otolith radius at age as dependent variable, and Age of sampling (Age), Feed, Density as fixed independent factors and Tank as a random independent factor nested in Feed and Density. Den. refers to denominator in degrees of freedom (Df) and mean squares (MS) estimations of random factors

	SS	Df	MS	Den. Df	Den. MS	F	*p*
Otolith radius at 36 dph							
Age	3556.8	2	1778.4	135.0	110.8	16.05	<0.0001
Feed	18379.7	1	18379.7	4.5	200.4	91.73	0.0004
Density	586.1	1	586.1	4.5	200.6	2.92	0.1543
Age*Feed	3152.5	2	1576.3	135.0	110.8	14.22	<0.0001
Age*Density	302.5	2	151.2	135.0	110.8	1.36	0.2589
Feed*Density	1405.2	1	1405.2	4.0	210.7	6.67	0.0608
Tank(Feed*Density)	845.0	4	211.2	135.0	110.8	1.91	0.1130
Error	14959.9	135	110.8				
Otolith radius at 43 dph							
Age	1815.2	1	1815.2	101.0	118.1	15.38	0.0002
Feed	23206.8	1	23206.8	4.6	225.1	103.10	0.0003
Density	906.4	1	906.4	4.5	225.2	4.02	0.1068
Age*Feed	1859.0	1	1859.0	101.0	118.1	15.75	0.0001
Age*Density	518.7	1	518.7	101.0	118.1	4.39	0.0386
Feed*Density	1228.5	1	1228.5	4.0	239.1	5.14	0.0857
Tank(Feed*Density)	958.7	4	239.7	101.0	118.1	2.03	0.0958
Error	11923.7	101	118.1				

in these groups at onset of weaning (Puvanendran et al. 2008) as well as a greater acceptance of formulated feeds in the R-groups (Koedijk et al. 2010). However, the relative size difference as measured by CV of length did not differ notably between groups. On the other hand, the co-feeding with natural zooplankton during the first week of weaning may have limited the occurrence of cannibalism during this period. There were no incidences of cannibalism in a previous study on Atlantic cod, when the larvae were weaned at an average size smaller than 10 mm (Baskerville-Bridges and Kling 2000a), and this corresponds with behavioural studies where cannibalistic behaviour was first observed in cod from 9 mm (Puvanendran et al. 2008). Most of the R-group larvae in this experiment were thus probably too small to engage in cannibalistic behaviour at the onset of weaning, while the Z-group larvae reaching 15–20 mm length were in the size range with the highest cannibalistic propensity (Otterå and Folkvord 1993; Puvanendran et al. 2008). Clear signs of cannibalism were observed among large individuals shortly after the experiment, and for a stricter comparison of the effect of feed type per se, the R-group fish should have been reared until they had achieved the same size as the Z-group fish.

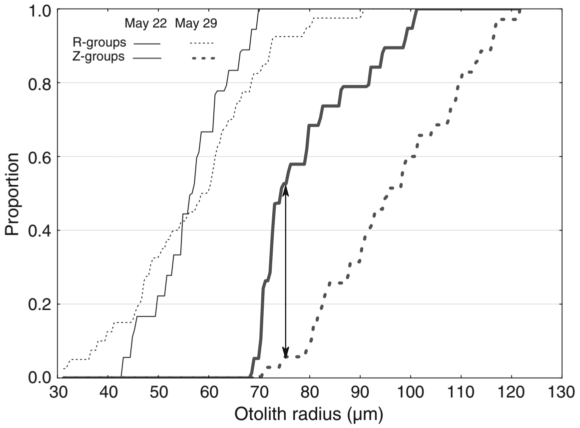

Fig. 4 Combined cumulative size distributions at May 22 (43 dph) of R-groups (*thin lines*) and Z-groups (*thick lines*). Data based on the May 29 sample in *dashed lines*, those from the May 22 sample in *solid lines*. The *arrow* indicates the proportion of fish in the Z-groups from the two sampling dates with otolith radii smaller than 75 μm at 43 dph

The higher overall mortality in the R-groups during weaning was not due to elevated size-selective mortality as revealed by otolith microstructure analysis of these groups. On the other hand, most of the overall mortality in the Z-groups was size-selective. In effect the apparent growth rate of the Z-group is therefore somewhat inflated towards the end of the experiment when estimated as changes in average population size-at-age due to selective removal of smaller individuals (Folkvord et al. 2009). Given sufficiently high sample sizes, selection curves can be estimated from the size distributions of the population prior to and after selection (Anderson 1995), but this could not be done on a per tank basis in this study. Caution is also advised on the combined data since few large specimens were present in the sample from the Z-groups on May 22. This may be a result of the relatively low sample size from May 22 (*n* Z-group larvae = 19), but could also be due to capture avoidance among larger individuals, which always remains a point of concern in selection studies (e.g., Meekan and Fortier 1996).

The comparison of survivors of different feeding regimes may not be straightforward in all cases. Survivors may represent a random (in a size context) subsample of the original population in one case, while in another case the survivors may represent a highly selected group of relatively large fish (e.g., Folkvord and Otterå 1993). The further comparison of the survivors from such groups should take this into account, and possibly compare the subsequent performance on a percentile basis of the original population. Under natural conditions size-selective survival of offspring has been documented in cod larvae and early juveniles (e.g., Meekan and Fortier 1996; Grønkjær and Schytte 1999), and in contrast to culture conditions the survivors only constitute a very small fraction of the initial population of newly hatched larvae. Still, a relatively high and selective loss of offspring during the juvenile fish production may represent a loss of genetic diversity which may impact later stages of the production cycle.

In conclusion, the otolith analysis revealed size-selective mortality that was not evident from the overall survival figures. Contrary to expectation, the groups with the highest overall mortality had the lowest extent of size-selective mortality. This is partly attributed to a fish size effect, where the smaller R-group larvae were less prone to cannibalistic behaviour than the Z-group siblings. The use of otolith studies in juvenile fish production is thus recommended where elucidation of mortality causes and patterns are of concern and possible selective survival is likely to occur.

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References

Anderson CS (1995) Calculating size-dependent relative survival from samples taken before and after selection. In: Secor DH, Dean JM, Campana SE (eds) Recent developments in fish otolith research. University of South Carolina Press, Columbia, pp 455–466

Baras E, Jobling M (2002) Dynamics of intracohort cannibalism in cultured fish. Aquac Res 33:461–479

Baskerville-Bridges B, Kling LJ (2000a) Early weaning of Atlantic cod (*Gadus morhua*) larvae onto a microparticulate diet. Aquacult 189:109–117

Baskerville-Bridges B, Kling LJ (2000b) Larval culture of Atlantic cod (*Gadus morhua*) at high stocking densities. Aquacult 181:61–69

Campana SE, Hurley PCF (1989) An age and temperature-mediated growth model for cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) larvae in the Gulf of Maine. Can J Fish Aquat Sci 46:603–613

Duffy JT, Epifanio CE (1994) Effects of larval density on the growth and survival of weakfish *Cynoscion regalis* in large-volume enclosures. Mar Ecol Prog Ser 104:227–233

Folkvord A (1991) Growth, survival and cannibalism of cod juveniles (*Gadus morhua* L.): effects of feed type, starvation and fish size. Aquacult 97:41–59

Folkvord A, Otterå H (1993) Effects of initial size distribution, day length and feeding frequency on growth, survival and cannibalism in juvenile Atlantic cod (*Gadus morhua* L.). Aquacult 114:243–260

Folkvord A, Johannessen A, Moksness E (2004) Temperature dependent otolith growth in herring (*Clupea harengus*) larvae. Sarsia 89:297–310

Folkvord A, Fiksen Ø, Høie H, Johannessen A, Otterlei E, Vollset KW (2009) What can size distributions within cohorts tell us about ecological processes in fish larvae? Sci Mar 74:119–130

Fox MG, Flowers DD (1990) Effect of fish density on growth, survival, and food consumption by juvenile walleyes in rearing ponds. Trans Am Fish Soc 119:112–121

Fox CJ, Folkvord A, Geffen AJ (2003) Otolith micro-increment formation in herring *Clupea harengus* larvae in relation to growth rate. Mar Ecol Prog Ser 264:83–94

Grønkjær P, Schytte M (1999) Non-random mortality on Baltic cod larvae inferred from otolith hatch-check sizes. Mar Ecol Prog Ser 181:53–59

Koedijk R, Folkvord A, Foss A, Pittman K, Stefansson SO, Handeland S, Imsland AK (2010) The influence of first-feeding diet on the Atlantic cod *Gadus morhua* phenotype: survival, development and long-term consequences for growth. J Fish Biol 77:1–19

Meekan MG, Fortier L (1996) Selection for fast growth during the larval life of Atlantic cod *Gadus morhua* on the Scotian Shelf. Mar Ecol Prog Ser 137:25–37

Mosegaard H, Folkvord A, Wright PJ (2002) V. Some uses of individual age data. B. Ecological applications. In: Panfili J, Troadec H, de Pontual H, Wright PJ (eds) Manual of fish sclerochronology. Ifremer-IRD coedition, Brest, pp 167–178

Nielsen R, Munk P (2004) Growth pattern and growth dependent mortality of larval and pelagic juvenile North Sea cod *Gadus morhua*. Mar Ecol Prog Ser 278:261–270

Otterå H, Folkvord A (1993) Allometric growth in juvenile cod (*Gadus morhua* L.) and possible effects on cannibalism. J Fish Biol 43:643–645

Puvanendran V, Laurel BJ, Brown JA (2008) Cannibalism of Atlantic cod *Gadus morhua* larvae and juveniles on first-week larvae. Aquat Biol 2:113–118

Shoji J, Tanaka M (2006) Growth-selective survival in pisciviorous larvae of Japanese Spanish mackerel *Scoberomorous niphoius*: early selection and significance of ichthyoplankton prey supply. Mar Ecol Prog Ser 321:245–254

Suthers IM, Sundby S (1993) Dispersal and growth of pelagic juvenile Arcto-Norwegian cod (*Gadus morhua*), inferred from otolith microstructure and water temperature. ICES J Mar Sci 50:261–270