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Calibrating and comparing somatic-, nucleic acid-, and otolith-based indicators of growth and condition in young juvenile European sprat (*Sprattus sprattus*)

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ABSTRACT

Reliable estimates of short- and longer-term in situ growth and condition of organisms are critical if one hopes to understand how the environment regulates survival. This study reports the first comparison of somatic- (*K*), biochemical- (RNA–DNA ratio, *RD*) and otolith- (increment widths, *OIW*) based indices of condition of a young juvenile fish. Measurements were made on European sprat (*Sprattus sprattus*) that had i) known differences in somatic growth rate caused by providing different, constant prey ration levels, ii) been fed ad libitum at 7, 11, 15, 18 and 22 °C, and iii) been deprived of prey for either 4, 8 or 12 days and re-fed for 8 days. All three proxies explained significant amounts (70 to 90%) of the variability in measured growth rate. In fish experiencing a change in their feeding level and concomitant change in mass-at-length (*K*), *RD* tracked changes in both length and mass while *OIW* only tracked changes in length. Values of *OIW* and *RD* were highest at 18 °C suggesting that this is the optimal temperature for growth in these juveniles. During food deprivation, *RD* and *OIW* rapidly decreased and reached their lowest values within ~4 days. Upon re-feeding, *RD* increased most rapidly, *K* was most variable and the response time in *OIW* was slowest (two-times slower than *RD*). These patterns reflected preferential allocation of food energy to restore body mass in recently re-fed fish prior to fish increasing both mass and length. These results indicate that the sensitivity and applicability of growth proxies depend on the recent feeding history, that proxies have different response times, and that caution be taken when inferring growth and condition in early life stages of fishes that forage in patchy prey environments.

1. Introduction

Gaining reliable in situ estimates of nutritional condition and growth status is a first step toward understanding how environmental processes affect survival and growth of organisms. In early life stages of marine fish, morphometric indices, biochemical measurements, and otolith microstructure analyses have been used to generate estimates of in situ growth and condition (e.g., Ferron and Leggett, 1994). The most common morphological index is the condition factor (*K*), a normalized metric of mass-at-length (e.g., Bolger and Connolly, 1989; Peck et al., 2005). The ratio of nucleic acid concentrations (RN–DNA ratio, *RD*) is one of the most commonly applied biochemical indices estimating recent in situ growth and/or condition of marine fish early life stages (e.g., Buckley, 1984; Malloy and Targett, 1994; Peck et al., 2003; Amara and Galois, 2004; Buckley et al., 2008; Meyer et al., 2012). Finally, otolith microstructure analysis has proved to be the most widely used technique to estimate individual growth histories (from measurements of increment widths) in young-of-the-year (larval and young juvenile) stages of marine fishes (e.g., Barkman and Bengtson, 1987; Jones, 1992; Oozeki and Watanabe, 2000). Each of these proxies (somatic, biochemical, otolith) can be affected by a number of intrinsic (e.g., fish size, developmental stage) and extrinsic (e.g., temperature, prey availability) factors (Ferron and Leggett, 1994; Suthers, 1998), hence controlled laboratory studies that examine the effects of these factors are required prior to interpreting measurements obtained on field-caught individuals.

Clupeid fishes are key players in the trophodynamic structure and function of many marine ecosystems such as upwelling and coastal shelf zones (Cury et al., 2000; Pikitch et al., 2012). Field studies examining in situ growth and condition of larvae and juveniles have often employed nucleic acid analyses (e.g., Chícharo et al., 1998; Ramírez et al., 2001) and/or otolith microstructure analysis (Butler, 1991; Bolz and Burns, 1996; Cermeñon et al., 2003; Rossi-Wongtschowski et al., 2003; Takahashi and Watanabe, 2004; Fey, 2005). Unfortunately, laboratory experiments on fishes in this family are relatively rare (e.g., De Silva and Balbontin, 1974; Folkvord et al., 1996; Opaliński

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et al., 2004; Peck et al., 2012, 2014), and experiments calibrating in situ growth proxies are largely lacking (although see Bernreuthr et al., 2013). This is also the case for sprat (*Sprattus sprattus* L.), a small pelagic clupeid important to both "top-down" and "bottom-up" processes in the Baltic, North, and Mediterranean Seas (Valenzuela and Vargas, 2002; MacKenzie and Köster, 2004; Peck et al., 2012). Measurements of otolith microstructure and nucleic acids have been made to assess growth and condition in sprat larvae in the field (Munk, 1993; Valenzuela and Vargas, 2002; Lee et al., 2006; Voss et al., 2006; Daenhardt et al., 2007; Günther et al., 2012). However, calibrations of somatic-, nucleic acid-, and otolith-based growth proxies have not been made mainly because of difficulties rearing sprat in the laboratory beyond the very early life stages. In contrast, young juvenile sprat can be easily captured from shallow coastal areas and acclimated to laboratory conditions (Baumann et al., 2005, 2007; Peck et al., 2012). The early juvenile period in Baltic sprat represents an important life phase when year class strength is ultimately determined likely via density-dependent processes such as top-down control of prey resources leading to starvation and mortality prior to or during the first winter of life (Baumann et al., 2007; Voss et al., 2012).

This study reports the results of laboratory trials conducted to evaluate and calibrate somatic-, biochemical-, and otolith-based growth proxies in early juvenile (29.5 to 57 mm standard length, L_S) European sprat. Specifically, measurements were made of *K*, *RD* and *OIW* of individuals from: i) 12-day feeding-growth trials conducted at 14, 18 and 22 °C, ii) 5-day ad libitum feeding trials conducted at 7, 11, 15, 18 and 21 °C, and iii) food deprivation and re-feeding trials (unfed for 4, 8 and 12 days) at 18 °C. All of these proxies take into account different, relevant aspects of growth physiology (catabolism and anabolism) and, given previous research, different expectations existed that could be tested. The first expectation was that growth rates established by feeding fish at different, constant ration levels would correlate well with each of these three proxies. Second, both *RD* and *OIW* were expected to reflect the thermal history of juveniles for well-fed fish at different (optimal and sub-optimally cold and warm) temperatures. Finally, food deprivation (re-feeding) was expected to cause most rapid changes in *RD* via immediate down- (up-) regulation in protein synthesis and slower responses in *K* and *OIW* via changes in allocation to mass and length (*K* and *OIW*).

2. Materials and methods

2.1. Laboratory fish rearing

Young juvenile sprat were collected by dip-net in a shallow coastal area at the mouth of Kiel fjord (54° 27′ N, 10° 11′ E), Germany. During August of 2003 and 2004, approximately 3000 to 5000 juveniles (25 to 30 mm L_S) were captured and immediately transported to the laboratory (IHF, Hamburg), where they were maintained in 150-cm diameter, flow-through tanks supplied with re-circulating seawater (mean ± range salinity = 15.0 ± 0.5). All experiments used a 14L:10D light regime with a daytime surface light intensity of ~4.0 μE s^{-1} m^{-2}. Fish were acclimated to different temperatures at a rate of 1 °C day^{-1} and maintained at test temperatures for at least one week prior to trials. Juveniles were provided a mixture of frozen copepods (*Cyclops* spp., SMF Aquaristik, Rheinzabern, Germany), newly hatched brine shrimp nauplii (*Artemia* spp. INVE Aquaculture, Brussels, Belgium) and artificial pellet diet (Start 700, Dana Feed, Horsens, Denmark) one or two times each day. Experiments commenced one to four weeks after field collection and were completed within four months, using the following sets of feeding levels and water temperatures.

2.2. Experiment 1: growth trials

Four laboratory feeding-growth trials lasting 10 to 17 days were conducted (Table 1). At the beginning of each trial, an equal number of fish was randomly loaded into each of 2 to 10 tanks and a random sample of 15 to 25 fish (Table 1) was chosen for initial measurements (see below). Within each trial, tanks were randomly assigned to one of four treatments: zero (no food), low, intermediate, or high (ad libitum) feeding levels. Brine shrimp nauplii (48 to 72 h post-hatch) were used as food. Fish maintained at low, intermediate and high (ad libitum) feeding levels received a ration (known number) of nauplii one, two, or four times a day, respectively, with each feeding event separated by at least 3 h. Tanks were static during the day and flow-through at night (>6 replacements), when uneaten food was collected in mesh bags positioned at tank outflows. Uneaten food was counted the following morning. The relationship between rates of food consumption and somatic growth will be presented elsewhere.

Trials were conducted at three temperatures: 14 ± 0.3, 18 ± 0.3, or 21 ± 0.4 °C. At the end of trials G1b–G4, all of the fish in each tank were measured (see below). At the end of Trial G1a, only half of the fish were sampled/removed from tanks and some of the remaining groups of fish were switched to a different feeding level in trial G1b (specifically, one tank was switched from high to low, one tank from low to high, and two tanks were switched from unfed to high). In trial G1b, the water volume of tanks was decreased from 135 L to 82 L to obtain the same stocking density of fish prior to and after fish removal. A total of 1015 fish was used in the four growth trials, and the mean mortality in tanks was low (~13%). Measurements of *RD*, *OIW* and *K* were made on fish in this trial (see below).

2.3. Experiment 2: temperature trials

Small groups of sprat (9–15) were maintained in each of four replicate tanks (diameter of 49 cm, volume of 54.5 L) under ad libitum feeding conditions for five to 11 days at 7, 11, 15, 18, and 21 °C (Table 1). Rations of *Artemia* nauplii were provided to fish using the same methods outlined in Exp 1 for high feeding groups. The sizes of fish were measured at the conclusion of the feeding trial. A total of 259 fish was used and mean mortality in tanks was ~4%. Both *RD* and *OIW* were measured in these fish (see below).

2.4. Experiment 3: starvation-re-feeding trial

A total of 50 fish was randomly loaded into each of six, 110-L circular (58-cm diameter), flow-through tanks (Table 1). Two replicate tanks were randomly assigned to one of three starvation–re-feeding regimes (unfed 4 days (U4), unfed 8 days (U8), or unfed 12 days (U12) and re-fed for 8 days). Three fish from each tank were sampled daily during the initial four days of food deprivation and re-feeding, otherwise sampling occurred every two days. Daily ad libitum rations of brine shrimp nauplii were used as food. Tanks had a mean water temperature of 18.6 (±0.1) °C. A total of 236 fish was sampled during the experiment and mortality was ~8%. Measurements of *RD* and *K* were made on fish from this trial (see below). Note, otolith increment widths were not measured in fish in this trial (Exp 3) but two groups of fish in trials G1a–G1b in Exp. 1 received the same 12-day food deprivation–re-feeding protocol.

2.5. Measurements

In each experiment, the salinity and temperature of each tank was measured daily (WTW Microprocessor Conductivity Meter LF 196, TetraCon 96-1.5 probe). The daily ad libitum ration was equal to the number of nauplii that could be reduced by 75 to 90% after a daily feeding period and ration levels were adjusted to accommodate for changes in fish size (growth) during the experiments. During each post-larval sampling in Exp. 1 (beginning and end of growth trial) and Exp. 2 (end of feeding trial) the individual mass (wet, M_W, and dry, M_D, ±0.1 mg, Sartorius 1773 MP8 balance ± 0.1 mg) and length (L_S, and total length, L_T, ±0.1 mm, caliper) were measured. The M_D was