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Growth Rates and Specific Aminoacyl-tRNA Synthetases Activities in *Clupea harengus* Larvae

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Abstract: Gaining robust in situ estimates of the growth rate of marine fish larvae is essential for understanding processes controlling year-class success and developing sustainable management strategies to maintain good environmental status. We measured the growth rate of Atlantic herring (*Clupea harengus*) larvae in the laboratory and compared it to the activity of aminoacyl-tRNA synthetases (AARS). Larvae were reared under controlled conditions for 20 days at three temperatures (7, 12, and 17 °C) using different prey concentrations (0.1, 0.3, and 2 prey·mL^{-1}) of the copepod *Acartia tonsa*. The relationship between specific growth rates (SGR) and specific AARS activities was best described by a linear function—SGR = −0.1031 + 0.0017 · spAARS, $r^2 = 0.71$, $p < 0.05$—when only larvae fed ad libitum were considered regardless of the temperature. When larvae fed with low concentrations of food were included in the analysis, the relationship was SGR = −0.0332 + 0.0010 · spAARS, $r^2 = 0.42$, $p < 0.05$. This latter slope was rather low compared to other studies performed in zooplankton. We suggest protein degradation during the early life stages of fish as the cause of this low slope. We also studied SGR under food deprivation and the effect on specific AARS activities. We found rather high specific AARS activities in small individuals of early stages of fish, also suggesting protein degradation. Further research about protein degradation and turnover rates is needed in order to use AARS activity as a proxy for growth rates in field-caught larvae.

Keywords: aminoacyl-tRNA synthetases (AARS); fish larvae; larval growth; protein degradation

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1. Introduction

Atlantic herring (*Clupea harengus*) is one of the most prolific and economically important fish species in the North Atlantic Ocean, playing a vital role both as a renewable marine resource and as a keystone species in marine ecosystems [1,2]. In 2021, catches of Atlantic herring in European waters alone reached approximately 1.3 million metric tons, underscoring its critical importance to commercial fisheries [3,4]. This fish is also an important component in the marine food web. As an example, herrings can exhibit top–down control on crustacean zooplankton in the Baltic Sea [5,6]. Recognizing the important role that Atlantic herrings play in marine ecosystems, knowledge about the growth dynamics of their larvae becomes imperative for predicting sustainable fishery production. Understanding growth rates in relation to bottom–up and top–down controls is crucial for sustainable fisheries' management and the preservation of the intricate balance within marine ecosystems.

The recruitment of Baltic Sea herrings, which occurs during the spring, depends on several processes influencing the growth and survival of early larvae [7]. While the growth

of the Atlantic herring was the subject of extensive studies [7,8], previous research explored larval growth rates using diverse techniques, including otolith micro-structure analysis [9]. Among the factor studied, temperature emerged as a critical element influencing larval growth rates [10,11], suggesting that the physical scenario explains a substantial portion of variability in growth during early developmental stages. However, existing studies primarily focused on a limited temperature range (8–12 °C), and time-series analyses by [12] revealed that 12–15 mm larvae typically experience considerably warmer temperatures (15 to 19 °C) in natural environments. Despite this, there are no laboratory studies investigating growth rates or calibrated growth indices for herrings at these higher temperatures. Addressing this gap is critical to improve our understanding of herring larval ecology and refine predictive models for recruitment.

Proxies for growth in fish larvae have traditionally centered on the RNA/DNA (RD) ratio, providing insights into cell protein synthetic capacity [13–16]. The RD ratio is valuable in assessing growth rates and condition in fish larvae [17–19]. Other biochemical indices such as lactate dehydrogenase (LDH) or citrate synthase (CS) show limitations, including time constraints, sample size requirements, and applicability to only specific larval stages [20]. The activity of aminoacyl-tRNA synthetases (AARS), the enzymes catalyzing the initial step of protein synthesis, has gained momentum as a growth proxy in various aquatic organisms. The activity of AARS correlates to growth rates in crustaceans such as *Daphnia magna* [21], *Calanus helgolandicus* [22], *Euphausia superba* [23], *Calanus finmarchicus* [24], *Oithona davisae* [25], and *Paracartia grani* [26]. However, studies performed in fish larvae using AARS as a proxy for growth rates are lacking.

In this study, we investigated the suitability of AARS activity as an index of growth in Atlantic herring larvae exposed to varying conditions. Specifically, our experiments included testing the growth response of larvae at different water temperatures and feeding on different prey levels, mirroring the conditions encountered by Baltic spring-spawning larvae in their natural habitat.

2. Materials and Methods

2.1. Sampling and Initial Acclimatization

Atlantic herring (*Clupea harengus*) eggs were collected from the Bay of Greifswald (Baltic Sea, northern Germany) using SCUBA divers. The eggs were retrieved from submerged aquatic vegetation at a water temperature of 17.3 °C and a salinity of 6.3. Subsequently, these eggs were transported to the laboratory and incubated under in situ conditions at a temperature ranging from 17 to 18 °C, and a higher salinity of 18. The decision to increase salinity was based on the necessity to ensure optimal larval survival under laboratory conditions, which may differ from natural environments. This change was observed to have no visible adverse effects on the eggs. After hatching, the larvae were transferred to rearing tanks and gradually acclimated to three experimental temperatures (7, 12, or 17 °C). Temperature and salinity were monitored daily during the experiments using a Cond 315i meter and TetraCon 325 Probe (WTW, Woburn, MA, USA). The experiments took place at the Elbe Aquarium facility (University of Hamburg, Hamburg, Germany). The temperatures used for the experiments were selected in relation to conditions that larvae may encounter in their natural environment, as well as potential scenarios reflecting environmental variability (7–17 °C). These temperatures included both suboptimal and optimal ranges for larval development. The increase in salinity to 18 aimed to match laboratory-rearing practices, ensuring a stable environment to study growth and feeding behavior [27]. While this change limited the direct extrapolation of the results to natural salinity conditions, it provided consistent experimental conditions.

2.2. Experimental Design

2.2.1. Larval Rearing

Larvae were reared under two feeding regimens: ad libitum feeding with a prey concentration of 2 prey·mL^{-1} [28] at 7, 12, and 17 °C, and limited feeding at concentrations

of 0.1 or 0.3 prey·mL^{-1} (12 °C only). Rearing occurred in six tanks with two replicate tanks for each temperature and prey level. Each 60 L tank was gently aerated, maintained with green water [29], and subjected to a 30% water exchange every second day.

Larvae (approximately 200 individuals) were fed daily with *Acartia tonsa* nauplii, with late nauplii and early copepodites provided as the larvae grew. Prey concentrations were verified prior to feeding. Sampling occurred every 2–3 days, with up to 10 larvae randomly collected per tank over 18 days. The larvae were photographed, measured for standard length (Ls ± 0.1 mm) from the tip of the mouth to the end of the notochord using an image analysis system (Optimas 6.0). Individual larval weight, in µg of dry weight (dw), was estimated from the length (mm) using the equation obtained by [30] from individual larvae sampled from the same tanks.

2.2.2. Food Deprivation Experiments

Food deprivation experiments evaluated growth indices under short- and medium-term starvation conditions: A short-term trial was carried out with larvae from the ad libitum treatment at 17 °C. The larvae were deprived of food in two replicate tanks, with a control tank for ad libitum feeding with *Acartia tonsa* nauplii as prey. A total of eight larvae per tank were sampled every 6 h for 36 h.

Medium-term trials were also conducted at 7 °C and 17 °C using three replicates per starvation treatment plus one control (fed ad libitum with *Acartia tonsa*). A total of twenty-one larvae per replicate were collected every 24 h for 5 days at 17 °C, or every 48 h for 6 days at 7 °C. During sampling, each larvae was photographed, transferred without water into a 1.5 mL Eppendorf vial, and immediately frozen and stored in liquid nitrogen (−196 °C) for biochemical analysis.

2.3. Growth Assessment

Specific growth rates (SGRs, d^{-1}) were calculated for each tank from the slope of the relationship between the natural logarithm of larval weight (µg dw·ind^{-1}) and age (days post hatch, DPH). SGR provides a reliable and sensitive metric for assessing growth dynamics across treatment groups.

2.4. Biochemical Assays

Frozen samples of individual larvae were homogenized in 500 µL Tris-HCl buffer (20 mM, pH 7.8) using an Eppendorf micropestle. Subsequently, the homogenates were centrifuged at 5000 rpm for 10 min at 0 °C. AARS activity was assayed at 25 °C following the method developed by [21], modified by [25], and adapted to microplate readings as described in [31]. Specifically, 50 µL of the sample supernatant was added to a mixture containing 40 µL of pyrophosphate (PPi) reagent (Sigma, Telde, Spain, P-7275) and 60 µL of Milli-Q water. The absorbance of the reaction mixture was monitored at 340 nm for 10 min using an SAFAS flx-xenius spectrofluorometer with microwell plates. The aminoacylation of the tRNA released PPi, inducing the oxidation of NADH. This oxidation was recorded as a decrease in absorbance (dAbs). The NADH oxidation rate (dAbs·min^{-1}) was then converted to the PPi release rate (AARS activity, nmol PPi·h^{-1}) using the equation provided by [32]. The AARS activity at the in situ temperature was obtained using the Arrhenius equation and the activation energy of 8.57 kcal·mol^{-1} given by [22]. We did not correct for the reduction in NADH by the electron transfer system, as [33] found that the NADH blank used to study this reduction was proportional to the specific AARS activity measured.

To obtain specific AARS (spAARS), we used the protein content as a measure of biomass. Proteins were determined from an aliquot of each homogenate using the Bicinchoninic acid method [34] with a Sigma-Aldrich protein assay kit (BCA-1 and B 9643). Bovine Serum Albumin (BSA) was used as the protein standard. The absorbance of the solution was measured at 562 nm using a SAFAS flx-xenius spectrofluorometer. Specific AARS activities were calculated dividing the enzyme activity by the protein content of each corresponding sample.

2.5. Statistical Analyses

Data analyses were conducted using STATISTICA 6.0 (Statsoft, Inc., Telde, Spain) and RStudio [35]. The effects of temperature and food level on dry weight (dw) and specific AARS activity were tested using a linear mixed-effects model, with random effect on replication, where the replicates were nested in temperature and food level treatment groups, respectively. The model for temperature followed the next form:

$$\log(\mathrm{dw}) = \beta_{0,7} + \beta_{0,12}I_{12} + \beta_{0,17}I_{17} + b_0 + (\beta_{1,7} + \beta_{1,12}I_{12} + \beta_{1,17}I_{17} + b_1)\,\mathrm{temp} + \varepsilon \quad (1)$$

where $b_0 \approx N(0,\sigma_0)$, $b_1 \approx N(0,\sigma_1)$, and $\varepsilon \approx N(0,\sigma\varepsilon_k)$. In this model, I_{12} and I_{17} are dummy variables such that $I_k = 1$ when the temperature is k degrees (k = 12, 17) and 0 otherwise. The terms b_0 and b_1 represent the random variation in intercept and slope due to the replicates. In this way, $\beta_{0,7}$ and $\beta_{1,7}$ represent the (reference) intercept and slope of log (dw) versus time when the temperature is 7 °C; $\beta_{0,12}$ and $\beta_{1,12}$ represent the variation in intercept and slope with respect to the reference values when the temperature is 12 °C; and $\beta_{0,17}$ and $\beta_{1,17}$ represent the respective variation with respect to the reference when the temperature is 17 °C. In the residual term ε, a heteroscedastic model was considered, with variance $\sigma\varepsilon_k$ depending on the temperature level and replicate. The same model was used for the different food levels. A significance level of $\alpha = 0.05$ was considered for the statistical analyses.

Additionally, the relationship between specific growth rates (SGRs) and spAARS activities, considering temperature and food concentration values, was analyzed using an analysis of variance (ANOVA). The Shapiro–Wilk test was used to assess normality, and the Levene test was used to assess homoscedasticity.

3. Results

The growth rates of *Clupea harengus* larvae during post-hatching feeding ad libitum (2 prey·mL^{-1}) at three experimental temperatures (7, 12, and 17 °C), including two replicates per experiment, showed significantly ($p < 0.05$) lower values at 7 °C and higher values at 12 °C (Table 1). However, the growth rates obtained a 17 °C were lower than at 12 °C (Table 1) and similar to the ones obtained at 7 °C (Figure 1). These values were estimated based on the aforementioned statistical model, which accounted for the random variability introduced by the replicates.

Table 1. Atlantic herring larvae specific growth rates (SGRs, d^{-1}) and specific AARS activities (spAARS, nmol PPi·mg prot^{-1}·h^{-1}) at different temperatures (°C) and food levels (prey·mL^{-1}); 'n' is the number of either individuals sized or samples analyzed (see Figure 1).

T (°C)	Food Level (prey·mL^{-1})	SGR (d^{-1}) (r^2, n)	spAARS ± SE (n) (nmol PPi·mg prot^{-1}·h^{-1})
7	2.0	0.06 (0.88, 90)	114.71 ± 13.87 (50)
7	2.0	0.06 (0.95, 89)	98.42 ± 7.91 (47)
17	2.0	0.09 (0.98, 153)	114.73 ± 8.71 (76)
17	2.0	0.07 (0.82, 220)	115.84 ± 11.11 (71)
12	2.0	0.17 (0.99, 75)	133.40 ± 16.27 (30)
12	2.0	0.16 (0.98, 75)	169.21 ± 19.36 (30)
12	0.3	0.08 (0.95, 75)	129.61 ± 8.40 (26)
12	0.3	0.11 (0.89, 75)	162.70 ± 10.77 (37)
12	0.1	0.14 (0.98, 59)	140.48 ± 9.38 (23)
12	0.1	0.11 (0.93, 45)	170.93 ± 13.71 (30)

Experiments performed with different food levels at 12 °C (Figure 2) showed no significant differences in growth rates between 0.1 and 0.3 prey·mL^{-1}. However, significantly higher growth rates ($p < 0.05$) were observed in larvae fed 2 prey·mL^{-1} (Figure 2c). These values were estimated based on the aforementioned statistical model, which accounted for the random variability introduced by the replicates.

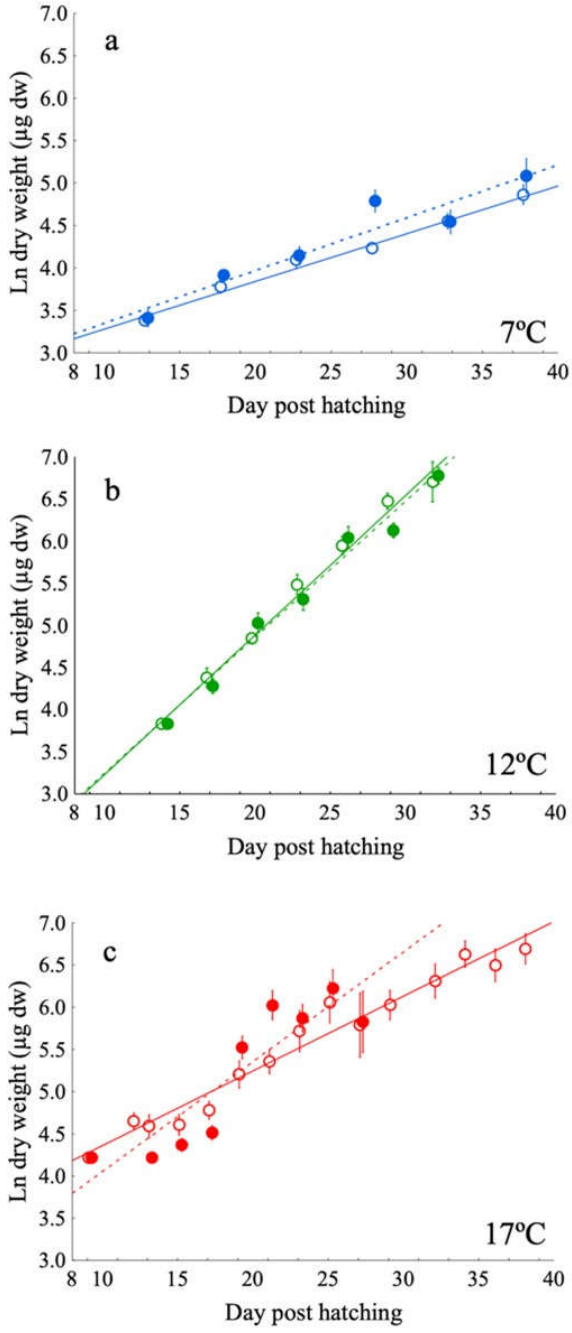

Figure 1. Changes in dry weight (Ln dry weight, µg dw ± SE) along the development of Atlantic herring larvae. (**a**) Results from two experiments at 7 °C, (**b**) two at 12 °C (2 prey·mL^{-1}), and (**c**) two at 17 °C. Specific growth rates (d^{-1}) were calculated as the slope of each regression line.

SGRs and specific AARS activities were compared at different temperatures (Figure 3a) and feeding levels (Figure 3b), showing similar trends between the two measurements. Both SGRs and specific AARS activities increased from 7 to 12 °C but decreased at 17 °C (Figure 3a). In contrast, no significant differences were observed in SGR values and spAARS activities across the three food concentration tested ($p > 0.05$, Figure 3b).

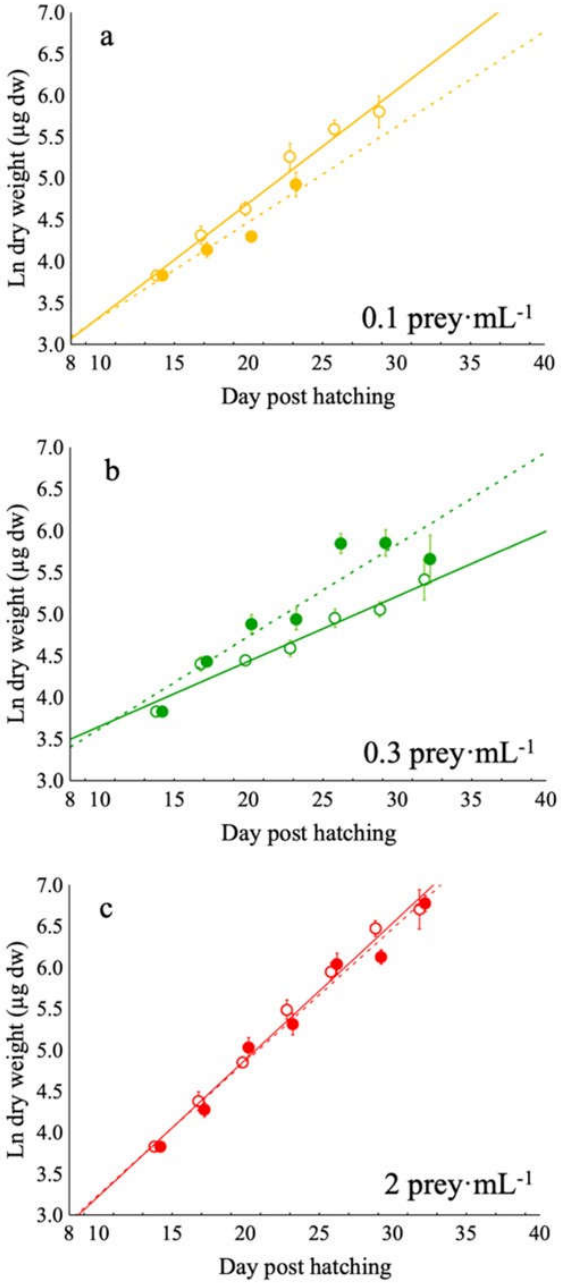

Figure 2. Changes in dry weight (Ln dry weight, µg dw ± SE) along the development of Atlantic herring larvae. (**a**) Results from two experiments at 12 °C (0.1 prey·mL^{-1}), (**b**) two at 12 °C (0.3 prey·mL^{-1}), and (**c**) two experiments at 12 °C (2 prey·mL^{-1}). Specific growth rates (d^{-1}) were calculated as the slope of each regression line.

A positive and significant correlation was observed between the specific growth rate (SGR, d^{-1}) and the average spAARS activity in larvae that had been fed ad libitum, regardless of the temperature to which they had been exposed (SGR = −0.1031 + 0.0017·spAARS, $r^2 = 0.71$, $p < 0.05$; Figure 4a). The existence of a positive correlation was also observed when regression was adjusted to include the larvae that had grown under low food concentrations (SGR = −0.0332 + 0.0010·spAARS, $r^2 = 0.42$, $p < 0.05$; Figure 4b).

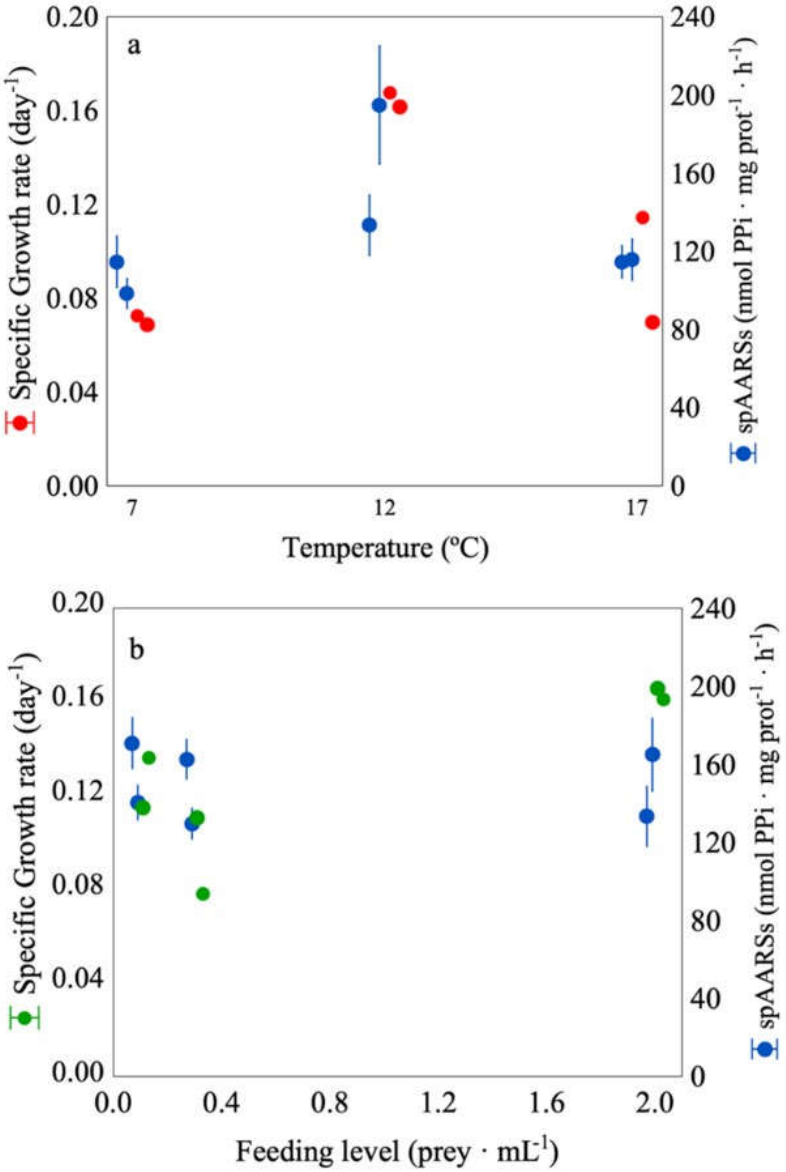

Figure 3. (**a**) Effect of temperature on SGR (day^{-1}) and spAARS activity (nmol PPi·mg $prot^{-1}$·h^{-1}) and (**b**) effect of food concentration on SGR (day^{-1}) and spAARS activity (nmol PPi·mg $prot^{-1}$·h^{-1}) at 12 °C.

Moreover, a negative exponential relationship was observed between specific AARS activity and individual biomass (µg protein·ind^{-1}; Figure 5). This pattern was consistent across various temperature conditions (Figure 5a) and under different food levels (Figure 5b). This result showed that smaller larvae, with an individual biomass below 200 µg protein·ind^{-1}, displayed the highest specific AARS values. In contrast, larger individuals showed lower and relatively stable specific AARS values.

Finally, the SGR of starved larvae ranged from −0.15 to 0.07 day^{-1} (Table 2). The relationship between the specific AARS activity and the individual biomass (µg protein·ind^{-1}) of unfed larvae showed a similar trend to the one observed for fed larvae (Figure 6). However, some low-weight individuals (<200 µg protein·ind^{-1}) displayed strikingly higher values of specific AARS than those in the experiments with larvae being fed at different prey levels or ad libitum.

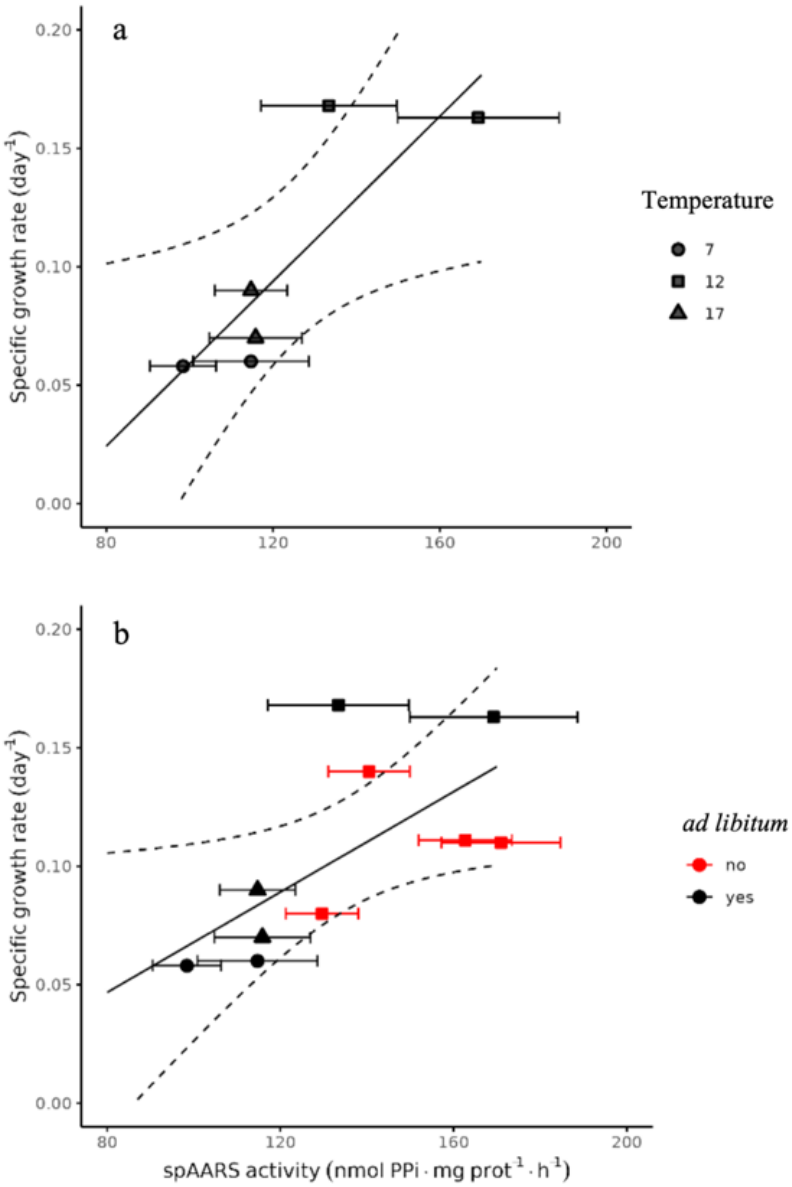

Figure 4. (**a**) Relationship between specific growth rates (day^{-1}) and spAARS activity (nmol PPi·mg prot^{-1}·h^{-1} ± SE), along with 95% confidence intervals, for larvae fed ad libitum at different temperatures. (**b**) The same relationship, now including Atlantic herring larvae growing under low food concentrations.

Table 2. Atlantic herring larvae specific growth rate (SGR, d^{-1}) and spAARS (nmol PPi·mg prot^{-1} · h^{-1}) under food deprivation; 'n' is the number of either individuals sized or samples analyzed. Data in italics correspond to experiments of a 36 h duration.

T (°C)	SGR (d^{-1}) (r^2, n)	spAARS ± SE (n) (nmol PPi·mg prot^{-1}·h^{-1})
7	0.01 (0.39, 46)	238.51 ± 20.90 (24)
7	0.01 (0.46, 43)	245.72 ± 24.49 (24)
7	0.01 (0.37, 56)	253.29 ± 20.35 (26)
17	0.07 (0.73, 73)	307.82 ± 20.98 (26)
17	*−0.15 (0.31, 60)*	*132.37 ± 12.91 (30)*
17	*0.05 (0.04, 64)*	*138.64 ± 17.29 (23)*

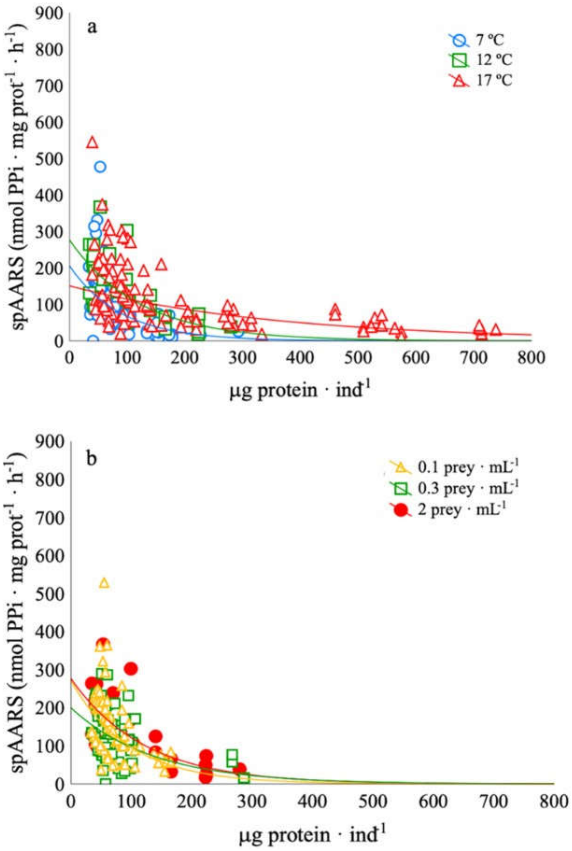

Figure 5. Relationship between specific AARS activity (nmol PPi·mg prot^{-1}·h^{-1}) and individual biomass (µg protein·ind^{-1}), (**a**) at different temperatures (°C) and (**b**) under different food concentrations (prey·mL^{-1}) at 12 °C.

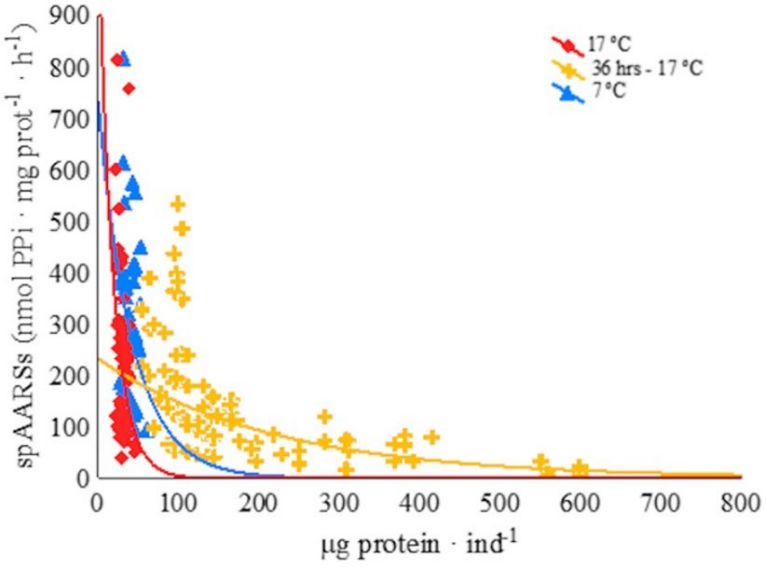

Figure 6. Relationship between specific AARS activity (nmol PPi·mg prot^{-1}·h^{-1}) and individual biomass (µg protein·ind^{-1}) under food deprivation.

4. Discussion

This study showed the relationship between specific growth rates (SGRs) and specific aminoacyl-tRNA synthetases (specific AARS) activities in Atlantic herring (*Clupea harengus*) larvae at different temperatures and food concentrations. The results revealed a positive correlation between SGRs and AARS activities under varying temperatures in organisms

feeding ad libitum. However, this relationship diminished under food-limited conditions due to the low variability in both SGRs and AARS activity under the different treatments. Interestingly, under food deprivation, the larvae showed high specific AARS activity despite low or negative growth rates, suggesting a mismatch between protein synthesis and body growth.

The specific growth rates (SGRs) observed in this study were consistent with those observed in previous experiments on this species [36–40]. However, the SGR at 17 °C was lower than at 12 °C, which agrees with studies indicating that temperatures above optimal levels could induce physiological stress and limit growth [41]. This is also in line with results by [42,43], who found how temperature-driven changes during early-life stages influence recruitment, showing the critical role of temperature for larval survival and growth. Ref. [44] also observed lower growth rates for herring larvae at 17.6 °C in the field, which was likely due to increased metabolic cost and enzymatic inefficiencies at higher temperatures. In contrast, the optimal growth rate observed at 12 °C corresponds to temperatures commonly experienced by larvae in their natural environment, reinforcing the idea that thermal stress can significantly impact growth beyond optimal ranges.

The similar SGRs observed at both 7 °C and 17 °C may reflect thermal limitations at both ends of the larvae's physiological tolerance range, where growth is constrained by suboptimal temperatures. Additionally, Ref. [39] observed a growth rate of 0.37 mm·d^{-1} at 17.5 °C in the field, which was similar to the maximum growth rate of 0.35 mm·d^{-1} observed at 8 °C in laboratory experiments [40]. Ref. [12] reported high growth rates at 17.5 °C using cohort analysis, which may produce biased values (over-estimates) of growth under conditions of the size-selective mortality of larvae. They suggested that the elevated zooplankton production in their study area could have contributed to the higher growth rates observed, which highlights the complexity of interpreting growth rate data in different environmental contexts.

Food availability also played a significant role in the observed SGRs. As expected, lower growth rates were recorded at low food concentrations in our study. The variability in growth rates, both in the literature and in our study, is substantial, with food concentration in the field being a key factor influencing these differences [37,38,40]. Moreover, at low food concentrations, we observed minimal variability in growth rates, suggesting that the larvae may have reached a saturation point where food availability no longer had a substantial impact on growth.

The most striking result of this study was the high AARS activity observed among starved *C. harengus* larvae, despite negative or minimal growth rates (Table 2). This counter-intuitive result was also observed in other marine organisms, including nauplii and adult stages of calanoid copepods [26,45]. Negative SGRs in starved *C. harengus* larvae were previously observed by [46], suggesting that protein degradation could lead to body shrinking. The negative SGR values in starved larvae were more pronounced in the early developmental stages than in juveniles, mainly because of the lower lipid reserves in juveniles [47,48]. During starvation, larvae lost weight primarily due to the increased metabolic cost of maintaining essential functions, with muscle mass being the only variable showing differences between the starved and fed organisms [14,49–51] due to fast protein degradation [52]. Our study indicated that, under food deprivation, larvae sustain high rates of protein turnover, but the proteins synthesized are rapidly consumed due to the lack of nutritional resources, rather than being accumulated as body mass. This phenomenon suggests that, under starvation, protein production is not directly transferred into growth, but rather into the maintenance of cellular functions and energy supply, leading to the observed mismatch between AARS activity and SGRs [53]. This mismatch showed the complexity of taking enzyme activity as the sole indicator of growth, especially under conditions of nutrient limitation.

The high values of specific AARS activity observed in small larvae, even under starvation, drives the lack of a strong relationship between SGR and AARS activity in the fish larvae observed in our study. An increased protein turnover rate under starvation is,

therefore, a challenge to relating SGRs and AARS activities, as organisms may display high AARS activity at low somatic growth rates. This should be further studied in organisms of different individual biomasses, growing at constant growth rates.

This pattern was also observed in *Paracartia grani* nauplii at low food concentrations [26]. These authors found high AARS activity in the early life stages of specimens with a low individual biomass, suggesting the existence of common patterns in the relationship between AARS activity and individual biomass. This fact could have significant implications for understanding growth processes in small-sized organisms, both in zooplankton and ichthyoplankton. High spAARS activities in young individuals under environmental conditions were significantly related to high growth somatic rates, whereas high spAARS activities under induced starvation would be due to the high protein turnover rates and the low accumulation of proteins in the larval body. Therefore, this fact could explain the mismatch between protein production and body growth observed in our study.

Comparing the correlations between SGRs and specific AARS activities in different published studies, two significant patterns were observed [31]. On the one hand, high slopes were found in *Daphnia magna* [21], *Euphausia superba* [23], and *Paracartia grani* nauplii [26], indicating a rather strong relationship between growth and AARS activity. On the other hand, rather low slopes were observed in *Calanus helgolandicus* [22], *Calanus finmarchicus* [24], and *Oithona davisae* [25] as depicted for *Clupea harengus* larvae. This variability was suggested to be related to organism size, with smaller individuals showing high AARS activity values more tightly linked to growth in larger organisms, where protein turnover is balanced with growth.

Implication and Limitations

The results indicated that specific AARS activities are a reliable proxy for growth rates under optimal conditions but become decoupled from somatic growth under starvation. This decoupling has significant implications for understanding the growth dynamics of marine organisms in natural ecosystems, where food availability and environmental conditions are highly variable. However, there are some limitations, such as the absence of field data to validate laboratory findings and the potential influence of prey type on enzyme activities and growth rates. Future studies should focus on incorporating field-relevant conditions such as natural food sources and explore how AARS activity and SGR are related across different species and developmental life stages.

5. Conclusions

Specific growth rates and AARS activities of *C. harengus* larvae were correlated under varying experimental conditions, although high AARS activities were observed under starvation. The results enhance our understanding of protein metabolism and growth regulation in marine fish larvae, providing insights into the physiological responses of ichthyoplankton to environmental stressors. However, the observed AARS-SGR mismatch under starvation highlights the need for further research to refine the use of enzymatic biomarkers in growth studies and explore the ecological implications of protein turnover in early developmental stages.

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