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On the edge of death: Rates of decline and lower thresholds of biochemical condition in food-deprived fish larvae and juveniles

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Abstract

Gaining reliable estimates of how long finfish early life stages can survive without feeding and how the rate of starvation and amount of time until death are influenced by body size, temperature and/or species is critical to understanding processes (e.g., predator-prey match-mismatch dynamics) governing mortality in the wild. The present study is a cross-species analysis of starvation induced-changes in biochemical condition in the early life stages of nine marine and freshwater finfish species. Data were compiled on changes in body size (dry weight, *DW*) and biochemical condition (standardized RNA-DNA ratio, *sRD*) throughout the course of starvation of endogenous and exogenous feeding larvae and juveniles in the laboratory. In all cases, the mean biochemical condition of groups decreased exponentially with starvation time, regardless of initial condition and endogenous yolk reserves. A starvation rate for individuals was estimated from discrete 75^{th} percentiles of sampling populations versus time [degree-days, $Dd = T$ (°C)*time (d)]. The 10^{th} percentile of *sRD* successfully approximated the lowest, life-stage specific biochemical condition (the edge of death). *T* could explain 59 % of the variability in time to death whereas *DW* had no effect. Species and life stage specific differences in starvation parameters suggest selective adaptation to food deprivation. Previously published, inter-specific functions predicting the relationship between growth rate and *sRD* in feeding fish larvae do not appear to apply to individuals experiencing prolonged food deprivation. Starvation rate, edge of death and time to death are viable proxies for the physiological processes under food deprivation in individual fish pre-recruits in the laboratory and provide useful metrics for studies on the role of starvation prevalence in the field.

Keywords

RNA-DNA ratio, starvation rate, mortality threshold, time to death, percentile approach

1. Introduction

The recruitment (year class) strength of marine fish species can vary by orders of magnitude between years (Houde, 1989) and is normally governed by processes that affect mortality rates during the first year of life (Houde, 2008). For example, changes in prey availability, resulting from temporal and spatial matches and mismatches of larvae and their prey, can alter larval growth rates and consequently the duration of the pre-recruit period – a period when larvae are particularly susceptible to mortality due to predators (Houde, 2008). Depending upon the degree of mismatch with prey, food-deprived larvae may die of starvation or weakened larvae may be more vulnerable to predation mortality (Skajaa et al., 2004). It is therefore critical to not only assess the degree of food limitation in the wild, but to also understand how the physiological process of starvation changes with species and/or life stage in order to gain a mechanistic understanding of the role that prey deprivation plays in recruitment processes.

For more than two decades, the nutritional condition of marine fish early life stages has been assessed using the ratio of nucleic acids (RNA-DNA ratio, *RD*) (Buckley, 1984; Buckley et al., 2008). The rationale behind the ratio is that RNAs are essential for the biosynthesis of proteins, while DNA levels in a cell remain fairly constant (Buckley et al., 1999; Bulow, 1987). Recently, an inter-calibration of *RD* measurements derived from different fluorometric protocols (Caldarone et al., 2006) has allowed for a multi-species comparison of protein-specific growth rates and *RD* in marine fish larvae resulting in a general model relating growth rate, and *RD* and temperature (Buckley et al., 2008). During food deprivation, declines in *RD* have been observed in exogenous feeding larvae of Atlantic cod (*Gadus morhua*) and Atlantic herring (*Clupea harengus*), reflecting the cessation of protein synthesis and somatic growth rates (e.g., Clemmesen, 1994; Grønkjær et al., 1997; Suneetha et al., 1999). Still, *RD* is not usually considered an indicator for starvation because some life stages and species can compensate for extended periods of food deprivation, either by catabolizing energy reserves within muscle and liver tissue or by utilizing embryonic yolk reserves, leading to ambiguous patterns in changes in *RD*. Direct means of describing and quantifying the phenomenon of starvation have included observing feeding success (gut content) (Bochdansky et al., 2008), measuring otolith microstructures (Johnson et al., 2002), and histological examination of digestive tissues (Kjørsvik et al., 1991; Theilacker and Watanabe, 1989). However, the potential influence of life stage and/or species relationships between nutritional deficiency, *RD*, and starvation in marine fish early life stages are not yet clarified.

Laboratory trials have contributed a wealth of knowledge on the factors and processes affecting larval growth and feeding and have often identified clear inter-individual differences in vital rates. Such phenotypic variability may arise from physiology (Peck et al., 2004) and/or genetic differences among the individuals (Case et al., 2006; Clemmesen et al., 2003; Saborido-Rey et al., 2003) and/or be exacerbated by behavioral interactions among individuals (Moran, 2007). Despite the best efforts to reduce this variability, laboratory reared groups often contain individuals having different nutritional or growth status. Inter-individual differences in growth potential are likely to cause differences in the responses of larvae to food deprivation (e.g., time to mortality, ability to re-establish feeding) but, to the best of our knowledge, this aspect of starvation response has remained unstudied. For example, laboratory trials normally use group mean values to describe the time course of changes in *RD* (Rooker and Holt, 1996; Suneetha et al., 1999; Tanaka et al., 2008). However, the resulting functional

model describing rates of starvation (e.g., rate of decrease in group mean *RD* versus time of food deprivation) will likely underestimate the rate occurring at the level of the individual.

Here, we propose an indirect method for addressing this problem based on the following assumptions: a) the condition of individuals at any point in time is stochastically distributed around a group mean value and b) the underlying function describing changes in *RD* with time of food deprivation is reasonably known (i.e. an exponential function) and c) larvae with a low condition suffer higher mortality rates than those in good condition (when starvation is the only source of mortality). Computing daily changes in the 75^{th} percentile of *RD* values appears to be a good option for estimating starvation rate since individuals within these upper percentiles represent a discrete sub-group of the whole population that is unlikely to change its relative ranking within the group on the short-term (Folkvord et al., 2009; Paulsen et al., 2009). Larvae in the upper percentiles will tend to survive the longest and will thus form an ever increasing portion of the population on subsequent sampling days (Figure 1), yielding a better approximation of the "true" starvation rate. The 90^{th} and 10^{th} percentile of the sampling population can be used as idealized start and end points, representing initial condition at onset of food deprivation and final condition close to starvation-induced mortality, respectively. We attempt to validate this "percentile approach" as a method to represent daily changes in *RD* at the individual level during food deprivation by comparing it to a conservative approach that utilizes group mean values.

Although individual variability will affect starvation rates, ambient temperature and body size will have profound effects on various aspects of metabolism, including energy losses due to routine respiration and excretion (Peck and Buckley, 2008; Peck et al., 2008). Protein synthesis and somatic growth rates are no exception to this rule and various methods for generalization and comparison have been suggested. For example, Buckley et al. (2008) reported that the best model describing instantaneous growth rates as a function of *RD* included a temperature interaction term (*T*x*RD*). Folkvord et al. (2005) assessed intra-specific differences of larval cod from two distinct populations with the aid of a coupled size-temperature-growth model while Malzahn et al. (2003) used degree-days to normalize the temperature dependence of somatic growth rates in North Sea houting larvae. The present study attempts to parameterize a common function that quantifies and, if applicable, normalizes the contribution of these two influential parameters on the change in biochemical condition (*RD*) of food-deprived individuals within controlled laboratory conditions. Our emphasis was to understand how intrinsic (body size, species) and extrinsic (temperature) factors contribute to differences in the response of individuals to starvation, including the rate of decrease and lowest (threshold) values of biochemical condition, and the time to death. Our results are discussed with respect to utilizing *RD* to help understand starvation-induced mortality in larval cohorts in the wild.

2. Material and Methods

2.1. Data set overview

We compiled previously published and unpublished data from laboratory-based, food deprivation experiments performed on nine species of marine and freshwater finfish larvae and juveniles, namely Atlantic cod, Atlantic herring, sprat (*Sprattus sprattus*), common goby (*Pomatoschistus microps*), southern flounder (*Paralichthys lethostigma*), vendace (*Coregonus albula*), North Sea houting (*Coregonus oxyrinchus*), haddock

(*Melanogrammus aeglefinus*), and sea bream (*Sparus aurata*). Detailed protocols and methods utilized in the different experiments can be found in the original publications (Table 1). The common feature of all experiments was that groups of well-nourished (either newly-hatched or previously *ad libitum* fed) fish were deprived of food for at least three days and samples (minimum of 5 fish per sample) were taken at the start and on at least two more occasions.

The data set included 3542 individually measured paired values of body size and RNA-DNA ratio (*RD*), stemming from 15 different experiments (Table 1). Experiments were partly made up of distinct trials using different ambient temperatures and/or initial body sizes and life stages (Table 2). Across all trials, water temperature ranged from 2.6 to 24.1 °C and body size (average initial *DW*) ranged from 35.9 µg in young larvae to 43.2 mg in juveniles. Endogenous feeding yolk sac larvae were used in 21 trials. Trial duration ranged from 2 to 36 days (Table 2). The end of each trial did not necessarily coincide with fish mortality. To be included, samples generated in an experiment had to be processed using only one technique for the measurement of body size and one single-dye fluorescence based protocol for determination of bulk nucleic acid contents. If not stated otherwise, *DW* was measured after freeze-drying to the nearest 0.1 µg (< 100 µg individuals) or 1.0 µg (individuals > 100 µg). In some cases, *DW* was calculated from known relationships with standard length [Experiment H, herring: Harrer (2006); Experiment N, southern flounder: Bolasina et al. (2006) and Qin et al. (2008)], protein content (Experiment D, cod, Experiment I, haddock: Caldarone, unpublished data), or wet weight (Experiment J, sprat: Peck, unpublished data).

2.2. RNA-DNA analysis

RD was measured in crude, whole body or muscle tissue homogenates using the non-specific, nucleic acid intercalating fluorescence dye ethidium bromide (Caldarone et al., 2001; Clemmesen, 1993; Suneetha et al., 1999; Wagner et al., 1998; Westermann and Holt, 1988). Subsequent addition of RNA- (and in some cases DNA-) specific restriction enzymes allowed the quantification of RNA and DNA in the same homogenate. In all protocols, standard reference materials (purified nucleic acids standards for RNA and DNA) were used to convert fluorescence yields into nucleic acid concentration. In cases where only DNA standards were used, the slope of the RNA standard curve was assumed to be 2.2 times lower than for DNA (Le Pecq and Paoletti, 1966). All *RD* values were standardized based on the assay-specific ratio of the slopes of the standard curves (DNA slope/RNA slope), standardized to a reference slope ratio of 2.4, as described in Caldarone et al. (2006). Standardized RNA-DNA ratios (*sRD*) will be used throughout the remainder of this manuscript. Assay-specific slope ratios and standardization factors are provided (Table 1).

Measurements of *sRD* were performed on either whole body or muscle tissue homogenates (Table 1). We intentionally abandoned any effort to convert muscle tissue *sRD* to whole body *sRD* realizing the unpredictable effects of differences in dissection protocols and potential differences in cell size (Olivar et al., 2009). However, early life stage muscle tissue *sRD* was previously shown to be affected by food deprivation and it does reflect the physiological process of starvation. To avoid potential bias, sample tissues types are indicted and measurements made on different tissues were treated separately in all calculations. We followed a similar approach for endogenous feeding yolk sac larvae (Table 2). The contribution of maternal RNA and yolk sac dry weight substantially affects *sRD* and estimates of body size, and are known to introduce a bias to growth model

estimates (Buckley et al., 2006). Yolk sac larval starvation parameters nevertheless provide a useful base for comparison of temperature- and species-specific physiological mechanisms of yolk mobilization.

2.3. Linearization of starvation rate

Following a percentile approach, starvation rates were calculated with regression models fit to the 75th percentile of each *sRD* sampling date versus the duration (days) of food deprivation. Visual inspection of linear and exponential model residuals confirmed the assumption of an exponential decrease in most trials (data not shown). In order to express starvation rates in a linear fashion, values of *sRD* were natural logarithm (ln-) transformed. If not stated otherwise, all analysis utilized ln-transformed 75th percentile *sRD* data. Only starvation rate parameters (decrease rate and time to death) from significant linear regressions were used for further analysis.

2.4. Temperature-normalization of starvation rate

A subset of data was used to validate the degree-day normalization of starvation rates (D: #12-14; F: #17-23; I: #40-42; K: #44-48; L: #49+50; M: #52+53; experiment and trial #, respectively, Table 2). Only trials with different rearing temperatures stemming from the same experiment were tested against each other to minimize uncontrolled effects (e.g., maternal/batch effects, body size and species differences). Time of food deprivation (days) was used as covariate in ANCOVA to test for significant differences between starvation rates at different temperatures (independent variable). The ANCOVA was repeated with temperature-normalized data, expressing time of food deprivation in degree-days (*Dd*). Temperature-normalization was considered successful when a significant effect of the independent variable in the original data became insignificant.

2.5. Body size and life stage effect on starvation rate

A subset of data was used to test the influence of body size and life stage on starvation rates after temperature-normalization (A: #1-5; B: #6-10; E: #15+16; G: #24-29; G: #30-35; H: #36-39; experiment and trial number, respectively, Table 2). Only trials with different initial dry weight stemming from the same experiment were compared to minimize uncontrolled effects (e.g., maternal/batch effects, species differences). Endogenous and exogenous feeding larvae were compared in 3 larval cod experiments (Experiment: A, B and E). Only one experiment (Experiment: G, herring) spanned a sufficient body size range to compare early exogenous feeding larvae with post-larvae. Time of food deprivation, expressed in degree-days, was used as the covariate and average initial dry weight as the independent variable in an ANCOVA.

2.6. Population percentiles and time to death

Time to death was calculated using the formula:

$$(1) \qquad time\ to\ death = \frac{\ln(sRD_{10th\ perc}) - \ln(sRD_{90th\ perc})}{starvation\ rate_{75th\ perc}}$$

where ln($sRD_{\text{10th perc}}$) and ln($sRD_{\text{90th perc}}$) represent the 10th and 90th percentile values of ln-transformed *sRD* for the sampling population and $starvation\ rate_{\text{75th perc}}$ represents the temperature-normalized starvation rate based on the 75th percentile of daily ln-transformed *sRD* (Figure 1). The 10th percentile of *sRD* values in any population

of food-deprived individuals with sufficient variability in condition is thought to be an approximation for the lowest level of biochemical condition sustaining life. A direct estimate for this level was difficult to extract from the present data set as most of the food deprivation trials ended before larval mortality (33 out of 55 trials), and the 75th percentile values of *sRD* calculated for the last sampling day of these trials may not be ultimately close to death. It was therefore assumed that the 10th percentile of the full sampling population (pooled over time) is a better approximation for the edge of death. The 90th percentile is thought to represent the highest observed species- and life stage-specific *sRD* and, in turn, provides a normalized start-value for the onset of food deprivation.

2.7. Validation of the percentile approach

In an attempt to validate the percentile approach, starvation rates based on the 75th percentile were compared to starvation rates calculated from the arithmetic mean of the population on each sampling day. Additionally, the starvation parameters '10th (edge of death)' and '90th (normalized start-value) percentile of *sRD*' were regressed against observed start- (day 0) and cut-off (final sampling day) values of condition. 'Time to death' (Formula 1) from trials ending with larval mortality (significant endpoint trials) was regressed against observed time to death (i.e. trial duration). Percentile-based and mean-based 'time to death' were regressed against observed trial duration. Only trials yielding a significant slope parameter estimate (for the respective mode of calculation) were included in this regression. Mean-based 'time to death' used the average sampling day *sRD* at the first and last sampling day as start- and endpoint, respectively. The 10th percentile *sRD* of trials using whole body tissue preparations was converted into somatic growth rates, using a multi-species, temperature corrected model describing instantaneous growth rates as a function of *sRD* (Buckley et al., 2008) to assess the validity of this metric for fish under food depletion.

All statistical calculations were performed with PASW Statistics 18 (SPSS Inc.). Criteria of normality, homoscedasticity, homogeneity of regression slopes and independence of covariate and treatment effects were respected for Analysis of Covariance (ANCOVA) and multiple regressions (Field, 2009). Significance level was set to $p \leq 0.05$. If not stated otherwise, means are given ± standard deviation, SD.

3. Results

As expected, *sRD* decreased with time of food deprivation in all 55 food deprivation trials. This was observed in whole body (*wb*) as well as muscle tissue (*mt*) sample preparations and in endogenous and exogenous feeding life stages. Examples of these trends for cod, herring and other species are provided (Figure 2-4). Starvation rates, i.e. significant linear regressions of the 75th percentile of ln-transformed *sRD* versus time (*Dd*) of food deprivation, were successfully calculated for 34 trials and ranged from -0.0206 $*Dd^{-1}$ in 17 dph exogenous feeding cod larvae to -0.0030 $*Dd^{-1}$ in newly hatched herring yolk sac larvae (Table 2). Muscle tissue starvation rates ranged from -0.0043 $*Dd^{-1}$ to -0.0050 $*Dd^{-1}$ and were therefore significantly higher (slower) than the average (-0.0091 $*Dd^{-1}$) whole body starvation rate (M-W test, U=5.0, z=-2.52, p<0.05). When looking at whole body sample preparations only, starvation rates of yolk sac larvae (-0.0089±0.0047 $*Dd^{-1}$) where not significantly different from exogenous feeding larval rates (-0.0093±0.0038 $*Dd^{-1}$, M-W test, U=97.0, z=-0.689, p>0.05). Average (absolute) starvation rates for species, ranked in the order *G. morhua* > *M. aeglefinus* > grand mean > *C. harengus* > *P. microps* > *P. lethostigma* > *C. albula* > *C. oxyrinchus* > *S. sprattus* [-0.0140(0.0043)

> -0.0131(-) > -0.0087(0.0041) > -0.0077(0.0021) > -0.0066(0.0007) > -0.0050(-) > -0.0048(0.0001) > -0.0045(-) > -0.0043(-)*Dd^{-1}, respectively]. Trials in which a significant regression slope could not be calculated (21 trials) were found across all species, temperatures and body sizes (indicating no bias).

3.1. Temperature-normalization of starvation rate

Temperature had a significant influence on the dependent variable (ln-transformed 75th percentile s*RD*) when time of food deprivation was expressed in days (not all analysis results shown, Table 2). Significant differences (ANCOVA) in comparison to the reference trial (highest temperature in the same experiment) were found in every experiment of the subset of data that was used for validation of the degree-day normalization, except for one trial at the lowest temperature of Experiment K (*P. microps*). This experiment spanned the broadest temperature range (14.3 °C) in a single species of the whole data set and the failing of the *T*-normalization was observed between the two extreme ends of the *T*-range. This indicates a potential limitation to the general applicability of this normalization-procedure when applied over such a broad range. All other experiments (and species) used a narrower *T*-range and it was therefore assumed that *Dd*-normalization would explain a significant proportion of temperature effects across the whole data set. Whereas temperature explained a significant proportion of variability in starvation rates before normalization (linear regression, *B*=-0.0056(0.0011), ß=-0.69, $p<0.001$, $R^2=0.48$; Figure 5A), it lost a great part of its predictive capacity after normalization (*B*=-0.0004(0.0001), ß=0.40, p=0.027, $R^2=0.16$; Figure 5C). The difference in explained variability (32% of the total variability) represents two thirds of the initially explained variability that was successfully channeled into *Dd*.

3.2. Body size and life stage effect on starvation rate

Body size was found to have a significant effect on starvation rate in four out of five experiments, but only when endogenous and exogenous feeding larvae were equally included in the analysis (Experiments A, B and E). In these experiments, only the starvation rate of yolk sac stages was significantly different (ANCOVA, Table 2) from the reference category (highest initial dry weight). When these life stages were excluded, no significant difference was found. From the two experiments that did not contain any endogenous feeding stages (Experiment G and H, both on *C. harengus*, dry weight range: 207.4 to 6051.1 µg and 215.6 to 537.5 µg, respectively) it can be hypothesized that around ~500 µg dry weight there is a breaking point for detectable body size difference in starvation rate. Before (Experiment H) and beyond (Experiment G) that point, body size had no effect on starvation rate. Exploratory data-analysis of starvation rates against body size (here: log dry weight) indicated a faint positive correlation between these two variables, not in original starvation rates (*B*=-0.005(0.009), ß=0.10, p=0.591, $R^2=0.01$; Figure 5B) but in *Dd*-normalized starvation rates (*B*=-0.002(0.001), ß=0.42, p=0.014, $R^2=0.18$; Figure 5D). When looking only at exogenous feeding larvae and whole body tissue preparations in the latter case, a significant proportion of variability in starvation rate could be explained by body size (*B*=-0.005(0.001), ß=0.67, p=0.002, $R^2=0.45$; Figure 5D).

3.3. Population percentiles and time to death

The 10th percentile of *sRD*, the approximation of the lowest biochemical condition sustaining life, spanned almost one order of magnitude (range: 0.3 to 2.3) across all species and life stages (Table 2) and was both variable across temperature (Figure 6A) and body size (Figure 6B). This variable was significantly correlated

with temperature when endogenous (*B*=-0.074(0.028), ß=-0.65, p=0.023, R^2=0.42) and exogenous feeding stages (*B*=-0.084(0.021), ß=-0.69, p=0.001, R^2=0.48) were separated. Whereas the slopes of these regressions were almost identical, the intercept for exogenous feeding stages was higher (2.4±0.3, estimator ± S.E.) than for endogenous larvae (1.7±0.3). A clear correlation with body size could not be found, but life stage specific trends were apparent for cod and herring larvae. On average, yolk sac larvae of these two species had lower 10^{th} percentile values (1.1±0.1, mean ± S.E.) than exogenous feeding stages (1.7±0.1), whereas life-stage independent averages were very similar (1.5±0.04 and 1.5±0.2). The 90^{th} percentile, the approximation for *sRD* at onset of food deprivation, ranged from 1.0 to 4.9 and was not correlated to water temperature, body size, species or life stage (Table 2). Time to death ranged from 33.4 to 409.7 *Dd* (Table 2) and was significantly correlated to temperature in both endogenous and exogenous feeding stages (Figure 7A), but not to body size (Figure 7B). A multiple regression using temperature and body size as predictors explained 59 % of the observed variability, but *DW* influence was insignificant (Temperature: B=10.284(1.632), ß=0.76, p<0.001, change in R^2=0.57; Dry weight: p=0.59). The effect of temperature on time to death in endogenous feeding larvae (*B*=11.719(1.797), ß=0.85, p<0.001, R^2=0.71) was not significantly different from that on exogenous feeding larvae (*B*=12.396(2.420), ß=0.85, p<0.001, R^2=0.72) as indicated by similar slopes of the respective regressions.

3.4. Validation of the percentile approach

Starvation rates based on the 75^{th} percentile were regressed against starvation rates calculated from the arithmetic mean of daily sampled populations (*B*=0.997(0.044), ß=0.97, p<0.001, R^2=0.95). The slope of the regression was not significantly different from 1 (CI range for slope estimator *B*: 0.907 to 1.088). The 10^{th} percentile (edge of death) of trials ending with larval mortality (22 trials, including endogenous and exogenous life stages, all tissue types) slightly overestimated the observed average *sRD* of the last sampling day, but was nevertheless significantly correlated to it (*B*=1.123(0.100), ß=0.93, p<0.001, R^2=0.86). The slope of the regression was not significantly different from 1 (CI range for slope estimator *B*: 0.914 to 1.332). The 90^{th} percentile (normalized start-value) of all trials (except for two outliers, both Experiment E) slightly underestimated the observed average *sRD* at onset of food deprivation (*B*=0.982(0.026), ß=0.98, p<0.001, R^2=0.96). The slope of the regression was not significantly different from 1 (CI range for slope estimator *B*: 0.929 to 1.035). Time to death, based on the percentile approach and calculated for all endpoint trials (ending with larval mortality), slightly underestimated observed time to death, i.e. trial duration (*B*=0.738(0.041), ß=0.99, p<0.001, R^2=0.97; Figure 8, Insert A). The slope of the regression was significantly different from 1 (CI range for slope estimator *B*: 0.645 to 0.831). Visual inspection of residuals showed the heavy influence of a single trial (#49, *C. oxirynchus*, *mt*, yolk sac stage) at the upper end of the regression. Time to death, both calculated from the percentile approach and the arithmetic mean, were regressed against trial duration (Figure 8 and Figure 8, Insert B, respectively). The percentile approach significantly underestimated trial duration (*B*=0.793(0.035), ß=0.97, p<0.001, R^2=0.94, CI estimator: 0.721 to 0.865), whereas the slope of the population mean based estimator was not significantly different from 1 (*B*=1.002(0.044), ß=0.97, p<0.001, R^2=0.94, estimator CI: 0.912 to 1.091). A linear regression with a slope of 1, representing 100 % congruence of the calculated and the observed metric, was added to the data and residuals were calculated. In only 5 out of 34 cases, the percentile approach metric showed a positive residual, supporting the assertion that the larvae in most of the trials were sampled slightly before they were ultimately close to mortality. In contrast, in 17 out of 36 cases the population mean based metric extrapolated time to death beyond the actual end of the trial. When 10^{th} percentiles of *sRD* were converted into recent,

instantaneous growth rates, this metric ranged from -0.065 to 0.071 $*d^{-1}$ in all food deprivation trials. In endogenous feeding stages, most of these calculated growth rates were negative (-0.026 in average, range from -0.063 to 0.008 $*d^{-1}$). In exogenous feeding larvae, the average growth rate was positive (0.007, range from -0.043 to 0.061 $*d^{-1}$).

4. Discussion

Natural variations in the abundance of fish stocks can be the result of several factors acting on all life stages. The fast growth and high mortality rates observed for larval fish have led researchers to the conclusion that processes acting during the larval stage have the potential to introduce major variability in the recruitment levels of marine fishes. The ability to gain robust in situ growth estimates and distinguish individuals that are growing well from those that are growing poorly is therefore critical (Houde, 2008). Starvation and predation are considered to be the most important causes of mortality in the early life stages of fish (Houde 1987; Bailey and Houde 1989). The combination of low ability to detect and escape from predators, and high metabolic rate and limited energy reserves, make larvae vulnerable to mortality via both predation and starvation (Houde, 1987; Fuiman and Cowan, 2003; Bochdansky et al., 2008).

The measurements of biochemical condition (*RD*) is one of the most widely used biochemical growth indicators for marine fish early life stages (e.g., Buckley, 1984; Buckley et al., 2008; Chícharo and Chícharo, 2008; Clemmesen et al., 2003) and recent work has standardized this ratio (s*RD*) allowing comparison of measurements made in different laboratories (Caldarone et al., 2006). In our study, we used a novel approach to examine changes in s*RD* in food-deprived individuals to 1) identify species- and body size-specific lower s*RD* (threshold) values, 2) quantify the rate of change in s*RD* in food-deprived individuals, and 3) estimate the time required to reach threshold values in the early life stages of nine different marine and freshwater fish species. These parameters are the corner-pillars of theories describing mortality via starvation in finfish pre-recruit life stages (Ferron and Leggett, 1994). We also demonstrated how inter-individual variability in s*RD* can be harnessed to provide better estimates of starvation trajectories by using a percentile approach and argue that the physiological rate of starvation is better estimated using this technique compared to traditional calculations that employ group mean values. The low s*RD* values of food-deprived individuals in the present studies translate into positive and negative growth rates, following the relationship described by Buckley et al. (2008). Our results therefore suggest that the published general model (Buckley et al., 2008) and species-specific models (e.g., Caldarone, 2005; Caldarone et al., 2003) explaining the relationship between growth rate and s*RD* in feeding fish larvae do not apply to individuals experiencing prolonged food deprivation. Although well-nourished individuals can exploit their growth potential within the boundaries determined by species and/or water temperature (Buckley et al., 2008), the effect of food deprivation seems to overrule any generic (inter-specific) relationship, replacing it by life stage- and species-specific adaptations.

4.1. Temperature-normalization of starvation rate

In the present study, temperature effects on starvation rates within species were successfully normalized by the degree-day metric. There was, though, an unexpected positive correlation between temperature and calculated time to death, which was meant to be a sufficiently temperature corrected metric. This is unusual because it means that at higher temperatures fasting larvae of similar size and life stage exhaust their energy reserves later.

This counter-intuitive result might indicate that degree-day normalization has its limits for this particular case. Although still not common, the normalization of poikilotherm life history to overcome differences in temperature has been shown to be extremely valuable (Fuiman et al., 1998; Neuheimer and Taggart, 2007). The basis for that is appealing: the metabolic rate of a poikilotherm depends upon the amount of energy transferred to the respective sites of metabolic activity, and metabolism is based on the same biochemical processes in a vast range of animals (Brown et al., 2004). For example, avian and aquatic poikilotherm egg development rates are highly temperature-dependent and species-independent when corrected for mass (Gillooly et al., 2002). The thermal history explained as much as 93% of the variability in length at age in 17 cod stocks (Neuheimer and Taggart, 2007).

In models examining the relation of *RD* to growth, including temperature as a variable greatly improved predictive power (Buckley, 1984; Buckley et al., 2008). Clearly, growth not only depends upon temperature, but also on the input of energy and nutrients (e.g., Peck et al., 2003). However, the majority of studies deal almost exclusively with positive growth rates (i.e. when rates of anabolism exceed catabolism) and both negative and positive growth rates are always examined in concert. This implies a basic assumption that there are no major physiological differences in growing or mildly fasting animals, even though this is usually not explicitly stated.

Recent research has unraveled systematic differences in thermodynamic trait response changes among representatives of all major biota and biomes (Dell et al., 2011). Whereas universal principles of thermal sensitivity (e.g., Boltzmann-Arrhenius model) were confirmed for autonomous traits (e.g., fundamental metabolic rates), somatic traits (i.e. more or less under conscious control) exhibited higher thermal sensitivity. Carefully translated to the early life stages of finfish, this suggests that foraging traits (e.g., swimming activity, prey ingestion and hence growth rates) are under lower selective pressure so that distinct thermal reaction norms and temperature optima are conserved across species and life stages. Opposed to this, predation-avoidance and other traits directly related to survival are under higher selective pressure, thus considerable energy is invested to maintain high trait performance across a broader range of temperatures. This suggests that somatic (behaviorally controlled) traits that have a life-saving function (e.g., searching for food to avoid death of starvation) might be correlated to autonomous (metabolic) traits and do not undergo selective pressure independently from one another. An example for such a somatic trait that exhibits thermal sensitivity, even under conditions of food deprivation (high degree of autonomous control), was described by Fukuhara (1990). Larvae were more active at warmer temperatures and changed their activity level in respect to the quantity of their remaining yolk reserves with a maximum activity level around the time of exhaustion.

4.2. Body size and life stage effect on starvation rate

Starvation rates were not universally correlated to body size, but body size could explain a significant fraction of variability when limited to exogenous feeding stages, and when ambiguous species-tissue type combinations were excluded. This rather weak relationship suggests that a) the ~2.5 order of magnitude difference in body size covered by the regression was necessary to yield a biological signal and b) that the species that were excluded from the regression either indicate a species effect or are an artifact of life stage (endogenous reserves) and tissue type. Our results further suggest that early stage exogenous feeding larvae are particularly vulnerable to starvation under laboratory conditions. After absorbing their yolk reserves, larvae must start first feeding within

a very limited window of opportunity (Miller et al., 1988). The window of opportunity is the time period between first feeding (closely related to mouth-gap opening and onset of foraging behavior) and the point of no return (Blaxter and Hempel, 1963). Overton et al. (2010) estimated the window of opportunity for Baltic cod yolk sac larvae in the laboratory at 10 °C to be 5.6 days (56 *Dd*). At warmer temperatures (19°C), Yúfera et al. (1993) estimated the window of opportunity to be only 2 days (38 *Dd*) for sea bream. It appears that mixotrophic larvae, those undergoing the transition from endogenous to exogenous feeding, rapidly deplete energy reserves when they are food-deprived. These young larvae have not yet had time to deposit energy-rich storage tissues (e.g., white trunk muscle or lipid rich liver) that increase their starvation resistance. Very short times to death for cod larvae at exactly this transitional life stage were determined in this study (on average, well below 50 *Dd*). Their rapid starvation rates as well as their highly variable condition in all trials were clear indicators of their pronounced vulnerability to starvation.

In sharp contrast to these vulnerable early stage larvae, juvenile sprat exhibited high starvation resistance and suggested an exception to the exponential decrease of condition with time of food deprivation. These individuals were food deprived for 18 days, but were not yet beyond their PNR because all fish were able to successfully re-feed and grow (Peck et al., in prep). The individual starvation rate trajectory indicates that this life stage has another strategy to respond to food deprivation. Other meta-analysis studies, investigating respiration rates in larval and later stage fish (Bochdansky and Leggett, 2001), show that metabolic control changes throughout ontogeny in a fashion that is not solely attributable to body size and storage tissue mass. After prolonged periods of starvation, clupeids (such as sprat and herring) continue to lose body weight and display decreasing somatic condition factors (*DW* per unit *SL*) yet only show very modest declines in *SL* (Hauss, 2008).

Due to the patchy distribution of their prey, their high growth capacity and high metabolic rates, post-larval and early juvenile fish must possess mechanisms to cope with periods of starvation (Wieser, 1991). Such mechanisms can include the preferential use of specific metabolic substrates (Arndt et al., 1996; Molony, 1993), changes in fish behavior including swimming activity and/or habitat use (Björnsson, 1993; Rudstam and Magnuson, 1985; Sogard and Olla, 1996; van Dijk et al., 2002) and down-regulation of metabolism (Mendez and Wieser, 1993; Wieser et al., 1992). Due to the costs and tradeoffs associated with these mechanisms (e.g., increasing foraging activity to increase the probability of encountering food versus the need to decrease metabolic costs to save energy), the strategy(ies) employed at any particular time appear to depend upon the magnitude of food deprivation (Sogard and Olla, 1996; Wieser, 1991).

We found an almost distinct break in relation to temperature between the edge of death (10^{th} percentile metric) in endogenous vs. exogenous feeding larvae. Using a multi-species model relating growth rate, and *RD* and temperature (Buckley et al., 2008), the condition of food deprived exogenous life stages was converted into growth rates that ranged almost equally from positive to negative, with an average just slightly above zero. In the case of the current data set, it was not possible to use this growth model, parameterized with well-nourished larvae from laboratory trials, to predict (negative) growth rates for food depleted larvae. When the same calculation was done for the 10^{th} percentile *sRD* of yolk sac larvae (i.e. when most of the endogenous reserves were already exhausted), growth rates were mainly negative, i.e. these life stages were more correctly identified to have a poor condition. Young yolk sac larvae (prior to any "mixed feeding period"), totally rely on their

endogenous reserves for growth and development. *RD* values are initially high in this life stage and have led to an overestimate of somatic growth rates using *RD*-temperature models that were successfully fitted to later stage larvae (Buckley et al., 2006). Besides potentially reduced net protein retention rates and RNA activity levels, the body weight (or protein-) specific growth rate of a yolk sac larva is heavily underestimated if yolk mass is not excluded from the calculation (Buckley et al., 2006). For this reason *RD* can be an indicator of recent growth in early yolk sac larvae when protein accretion in the larval body is considered independent from weight loss due to yolk absorption. For the whole larval body including the yolk reserves, maternal effects are crucial. Egg size and quality determine the quantity and composition of yolk reserves that provide the initial protein synthesis machinery (e.g., maternal ribosomal RNA) (Clemmesen et al., 2003; Saborido-Rey et al., 2003) and, therefore, have a profound impact on starvation resistance.

A special case among yolk sac life stage strategies was described for North Sea houting by Malzahn et al. (2003). The authors determined hyperplasia, i.e. the increase of cell numbers rather cell size, to be the main mode of growth up to 250 degree-days post hatch. Using the same muscle tissue *sRD* data set, we could demonstrate in the present analysis that these endogenous feeding houting larvae exhibited a) the lowest condition threshold (10^{th} percentile) which was presumably caused by the low RNA concentration in the hyperplasia muscle tissue and b) the highest time to death which was only possible due to fueling of extensive (maternal) yolk reserves. Houting and vendace, the only freshwater representative in the data set, belong both to the family of salmonidae, which usually have a large size at hatch and big yolk reserves. This allows them to accomplish a greater proportion of early ontogeny by feeding on endogenous reserves. The hyperplasia mode of growth is very efficient for the representatives of this life history strategy because it does not require any external feed intake for ontogenetic progress. This can be regarded as an adaptation to spawning and growing in a low temperature and/or food limited environment, where it is better for a larvae, besides having a big size at hatch, to be far developed when either temperatures or food abundances rise later in the season. Vendace larvae for example are spawned in winter-time and egg development takes place over the course of five months (3 °C, laboratory conditions) (Karjalainen et al., 1991).

Larval stages in the present laboratory studies exhibited some degree of body size and (early) life stage dependent differences in starvation rate and thresholds, as outlined above, but the ability to resist starvation remained generally low over orders of magnitude in body size. Folkvord et al. (2009) suggested a trade-off between energy storage and growth for the fast growing early life stages, where growth rate is more important to evade predation risk than to invest in starvation resistance. Miller et al. (1988) proposed a universal (72 species of marine and freshwater fish) relationship between size-at-hatch and numerous traits ascribed to larval foraging capacities and starvation susceptibilities. Jordaan and Brown (2003) also identified clear tradeoffs between body size, growth performance and starvation resistance. In their laboratory study, ~12 mm SL cod larvae had the highest potential for growth as well as starvation-induced mortality, while, in contrast, first-feeding larvae had a higher potential to withstand periods of prey deprivation (Overton et al., 2010). Other studies conducted on marine fishes (e.g., Bochdansky et al., 2008) reveal that larvae may need to pass through multiple “critical periods” where starvation resistance and growth capacity may be linked. In lieu of size-at-stage, utilizing an ontogenetic index that defines the developmental stage relative to metamorphosis (Fuiman et al., 1998) will

allow species-specific differences to become easier to interpret, as well as body size effects across species, since the same body size may be correlated to vastly different developmental stages among species.

Different tail and trunk muscle sections were used for *sRD* analysis in some of the experiments. Olivar et al. (2009) systematically investigated the different contributions of tissue type *RD* (e.g., head, eyes, muscle, gut) to whole body *RD* and found that muscle tissue had consistently higher *RD* values then any of the other tissues. This signal was conserved for two distinct life stages (pre- and post-flexion larvae) of two clupeid species and throughout laboratory food deprivation trials in pleuronectiformid larvae (Olivar et al., 2009). Muscle tissue is the most important energy storage for the late pre-metamorphosis life stages prior to lipid storage. Muscle growth is highly correlated to *sRD* because of its high protein synthesis rates. At the onset of food deprivation, protein turn-over rates in muscle tissue decrease and protein reserves are mobilized to satisfy catabolic needs, as suggested previously in starving fish larvae based on histological or cell-cycle analysis (Catalán et al., 2007; Catalán and Olivar, 2002). This process goes along with a reduction of ribosomal RNA and an increase of DNA content per unit dry weight (Bergeron, 1997). Therefore, the starvation signal can be found in this tissue type even though it presumably reflects only part of the physiological process in whole body (larval) samples. For the four species investigated by Olivar et al. (2009), the authors suggest a correction factor for tissue type effects. It still remains to be clarified how the change in the relative DNA content per dry weight throughout ontogeny, caused by the formation of low-DNA content organic matter like bones and lipid storages (Suthers, 1998), is differentially effected by species with different morphometric growth strategies (Froese, 1990).

4.3. Population percentiles and percentile approach

A key premise of this study is that inter-individual variability in growth potential and starvation resistance strongly determines cohort responses to environmental conditions. It is necessary to move beyond simply using group averages to characterize a cohort, both in the laboratory and in the field, as this metric does not accurately capture the heterogeneity typically exhibited by fish early life stages. If we are to derive predictive models of how larval condition relates to survival and growth at later life stages, we need to better define the distribution of the characteristics of individuals, such as values of condition indices, starvation resistance, and growth potential.

Monitoring individual larvae over time within a mixed population, even under controlled laboratory conditions, is extremely difficult, however, inferential methods can be developed such as those of Folkvord et al. (2009), who used cumulative size distribution to derive sub-population specific growth rates. In this case, a change in the cohort weight distribution over time suggested a higher mortality in smaller, slower-growing individuals. The rationale behind this approach is that the relative size of a larva compared to other members of the same cohort is not likely to change in the short term (see Paulsen et al. (2009), cod rank order stays from yolk sac to metamorphosis). Thus the comparison of larval growth among a certain proportion of the population is best achieved by comparing larval size from the same percentile from consecutive samplings rather than comparing any larval size against the mean size in the previous or following sampling.

The same may also be the case for larval condition, where condition at a given day to some extent will depend on the condition of the previous day. In the case of *RD*, the change in RNA content of a larva is likely to be more important for the short term change in biochemical condition since the total DNA content, reflecting the number

of cells in an organism, is not going to drastically change from one day to the next (Clemmesen, 1994). Further support for this assertion comes from a laboratory grading trial of Moran (2007), where slower growing juvenile *Seriola lalandi* were found to have a consistently low nucleic acid-based condition, growth and survival index, irrespective of the environmental conditions.

Direct experimental determination of an individual's condition trajectory under food depletion is virtually impossible to measure in larval fish. Ferron and Leggett (1994) proposed a conceptual model for various condition proxies (morphological, histological and biochemical) under recurring feeding and starvation conditions. In their sense, condition responds to changes in feeding regime within boundaries defined by *ad libitum* feeding and food-depletion. Depending upon the characteristics of the utilized condition proxy (e.g., responsiveness, sensitivity), the direction of change in individual condition may not always be apparent. This is especially true for *RD* that reflects the *ad hoc* status of protein synthesis rate and recent growth rate, but never the direction of change in condition with time.

The percentile approach is a first step to overcome the above mentioned inability to gain individual starvation trajectories and provides a metric that is otherwise not available from population means. It has to be realized though, that in the current study the same data set was used for development and application of this approach. Because these two subsequent steps are nested in each other, careful validation is mandatory. Because independent data sets were lacking and cross-validation (e.g., step-wise exclusion of individual experiments and validation against the remaining ones) would have required substantially more data, we compared the percentile-based starvation parameters with the conservative approach of population mean-based estimates. For the case of starvation rates, we found that these two parameters were almost identical. This indicates that the percentile- and mean-based regression slopes of condition against time of food deprivation were parallel in most trials and were only slightly off-set (higher intercept in 75th percentile). Most of the experiments applied sampling schedules that were designed for pooled estimates from replicate tanks, and presumably sampled just as many individuals from each tank as would be needed to get a "good" arithmetic mean. An increase in predictive power of the percentile approach can be expected when sampling size is increased until the 75th percentile is stabilized, i.e. changes discretely over time of food depletion. Both the 10th and the 90th percentile of the whole sampling population proved to be valid approximations of larval condition at onset of food deprivation and the edge of death. Transferring this finding from a tank-population in the laboratory to a field population, it is possible to circumvent labor-intensive identification of the duration of starvation time in individuals [e.g., from otolith increment widths (Baumann et al., 2005) or histology (Ehrlich et al., 1976; Gisbert and Doroshov, 2003)]. The calculated time to death was successfully validated with a rigid selection of trials that ended with larval mortality after removing one outlier trial (muscle tissue sample, yolk sac stage, Coregonid). For all other trials, time to death, based on the percentile approach, slightly underestimated observed time to death (or more correctly: trial duration) which would be well expected, because the majority of individuals were not ultimately close to death. These trials were stopped because of pre-determined experimental schedules and presumably the real edge of death would have been reached later and with a lower 10th percentile of *sRD*. Opposed to this, the population mean-based calculation overestimated time to death in the majority of the cases and this systematic error would be even more pronounced when the real edge of death would have been included. We therefore conclude for

these laboratory experiments that the percentile approach has been successfully validated and that it can provide an accurate approximation of starvation parameters at the level of individuals.

Whereas starvation rates can be readily determined under laboratory conditions using the percentile approach, it is mandatory to extract the two other metrics (10^{th} and 90^{th} percentile) from field populations, because they are ultimately linked not only to species and life stage, but also population and habitat characteristics. These two metrics have been previously applied within fisheries oceanographic research examining marine fish early life stages. Clemmesen et al. (2003) reported that the distribution of *RD* values and patterns of percentiles of *RD* were largely influenced by environments experienced by individuals during early life. Individuals in a mesocosm with warmer temperatures and higher prey concentrations had higher larval growth rates, higher *RD* values, and relatively large prey in their gut compared to individuals in a second mesocosm that was colder and had lower prey concentrations (Busch et al., 2009; Clemmesen et al., 2003). Moreover, the 10^{th} percentile value of *RD* was stable with time in the former mesocosm, indicating a non-selective environment (predators were absent) whereas it rapidly increased during the first three weeks in the latter mesocosm, suggesting that only the fittest larvae were able to survive and successfully compete for food in a prey-limited environment. Assessing changes in specific percentiles of *RD* has also yielded insight into how poor feeding and starvation may act to control early life stage survival in the field. Working on sprat larvae in the Bornholm Basin (Baltic Sea), Voss et al. (2006) reported that stable and relatively high 10^{th} percentile values of *RD* with increasing body size reflected a feeding environment that was best (high nauplii concentrations) for small, first-feeding sprat larvae in April and May. Lower 10^{th} percentile values of *RD* were found for the same sizes of larvae in July, suggesting a less selective environment (where both fast and slow growers survived). In the earlier time period, larvae in lower nutritional condition were removed from the population (i.e. suffered higher mortality), as reflected by a rise in the 10^{th} percentile. A similar increase in the 10^{th} percentile was observed by Huwer et al. (2011) who examined the nutritional condition of Baltic cod larvae during a period of prey limitation. The results of these studies are in line with a hypothesis originally proposed by Meekan and Fortier (1996), whereby during periods of abundant food supply, the selective pressure for fast growth is relaxed and slow-growing larvae may survive in larger proportions.

Perspective

An empirical parameterization of the functional model describing condition changes under food-depletion is instrumental in understanding the bigger picture of starvation prevalence and to answer the questions: When and where are early life stages of finfish pre-recruits exposed to starvation in the field? Is starvation an important cause for mortality? Köster et al. (2003) reported that mortality experienced during the larval stage was critical for recruitment success in Baltic cod. Model results of Hinrichsen et al. (2002) suggest that lower survival of Baltic cod larvae during the summer was due to prey limitation. In a recent field study, Huwer et al. (2011) reported Baltic cod larvae in poor nutritional condition within areas having low concentration of their preferred prey, *Pseudocalanus acuspes*. Working in the Northwest Atlantic, Buckley et al. (2010) identified 'windows for survival' for Atlantic and haddock larvae based upon seasonal and inter-annual differences in larval condition and prey abundance. Similarly, Voss et al. (2006) described size-specific 'windows of survival' for Baltic sprat larvae that were linked to the availability of suitable prey. These and other studies support Cushing's 'match–mismatch' hypothesis (Cushing, 1974, 1990) which outlines the key role of prey limitation during the early life

stages as a regulating mechanism of recruitment in many marine fish species. Thus, our efforts to quantify starvation rates and times to death based upon thresholds in biochemical indices (RNA-DNA) often measured in field programs should help advance our understanding of how prey limitation acts to affect early life stages of different species in the field.

To further improve this estimator, more research is needed on the point of no return (Blaxter and Hempel, 1963; PNR). This critical threshold is theoretically the only relevant true "edge of death", but it has only been examined in a handful of species and life stages and, thus, the literature is lacking estimates of larval condition that can be directly related to mortality (Chícharo, 1998; Lough and Mountain, 1996; Tanaka et al., 2008). The link between PNR and our *sRD*-derived time to death estimates is unclear, and given the recent restrictions in the types of experiments permitted due to animal welfare concerns (Huntingford et al., 2006), indirect measures of larval mortality risk may be the best available option. The advantage of the present *sRD*-derived measure of mortality risk is that it may be applicable as a first estimate across a wide range of species. The high rates of larval mortality in the field may also render the exact determination of PNR less relevant since most of the severely emaciated larvae will die even if some of them theoretically still would have the potential to survive if the food conditions abruptly improved.

Conclusion

The present study has shown that starvation-induced changes in condition in early pre-recruit life stages can only be described by a common function when temperature effects are adequately corrected for and life stage and species-specific differences are taken into account. Starvation rates were normalized with respect to temperature by expressing the duration of food deprivation on a degree-day basis. We fitted functional models to discrete percentiles of sampled populations to derive an estimate for starvation rates and mortality thresholds of biochemical condition in an individual. Within narrow ranges of body sizes and life stages, we were able to quantify key aspects of starvation (initial condition, rate of decrease, mortality threshold and time to death) based on data collected during controlled laboratory conditions. Although the selective loss of individuals in poor condition will undoubtedly differ between the laboratory and field, our analysis represents a step towards a tailored condition index that takes into account species-, stage- and time frame-specific attributes of starvation (Suthers, 1998). Additional research is needed to address gaps in our knowledge on how different life stages and/or species are able to cope with periods of prey deprivation and how often cohorts experience life "on the edge of death" in the wild.

Table 1 literature.

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(Caldarone et al., 2006; Caldarone et al., 2001; Clemmesen, 1993; Suneetha et al., 1999; Wagner et al., 1998; Westermann and Holt, 1988)

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819 **Figure captions**

820 **Figure 1:** Conceptual illustration of the percentile approach. A: Field populations contain at any time individuals
821 under food deprivation. Condition is a function of time of starvation and is stochastically distributed around
822 unknown mean values for each time-step of starvation. B: Condition in field population is stochastically
823 distributed. Population sample percentiles are used to describe the shape of the underlying distribution. In this
824 example, the population contains a high number of individuals in low condition; hence the lower percentiles are
825 closer to the population mean than the higher percentiles. C: Individual starvation trajectories of 5 individuals
826 from the population in A on the course of starvation over 4 time-steps. Slopes of starvation trajectories are
827 parallel for each individual a-e. D: Population percentiles of an experimental sampling population under food
828 deprivation. The 75^{th} percentiles of each sampling time-step x_i decrease with a similar slope as individuals a-e (in
829 C). Population percentiles represent highest (90^{th}) and lowest (10^{th}) possible condition under these environmental
830 conditions.

831 **Figure 2:** Untransformed data overview for cod (*Gadus morhua*) food deprivation trials. Standardized RNA-
832 DNA ratio of individuals is plotted against time of food deprivation (days). Black bars indicate the presence of
833 endogenous reserves. T – temperature (°C), BS – body size/dry weight (µg), YS – yolk sac stage. Panels are
834 arranged by trial (#) numbers (Table 2).

835 **Figure 3:** Untransformed data overview for herring (*Clupea harengus*) food deprivation trials. Standardized
836 RNA-DNA ratio of individuals is plotted against time of food deprivation (days). Black bars indicate the
837 presence of endogenous reserves. T – temperature (°C), BS – body size/dry weight (µg), YS – yolk sac stage.
838 Panels are arranged by trial (#) numbers (Table 2).

839 **Figure 4:** Untransformed data overview for food deprivation trials in seven species. Standardized RNA-DNA
840 ratio of individuals is plotted against time of food deprivation (days). Black bars indicate the presence of
841 endogenous reserves. Samples were either analyzed as muscle tissue (indicated) or whole body (not indicated)
842 homogenates. T – temperature (°C), BS – body size/dry weight (µg), YS – yolk sac stage. Panels are arranged by
843 trial (#) numbers (Table 2).

844 **Figure 5:** Starvation rate (significant linear slope of 75^{th} percentile ln-transformed *sRD* against time of food
845 deprivation) before (d^{-1}; A + B) and after (Dd^{-1}; C + D) degree-day-transformation plotted against water
846 temperature (°C; A+C) and log-initial dry weight (log(µg); B+D). Symbols indicate species (see inlet figure); *mt*
847 indicates muscle tissue *sRD* assays. Inserts: The same plot with symbols indicating endogenous (white fill) and
848 exogenous (black fill) life stages.

849 **Figure 6** Threshold *sRD*. 10^{th} percentile values of *sRD* in each trial are assumed to represent the lowest possible
850 biochemical condition sustaining life and therefore the edge of death. Threshold *sRD* for each trial plotted
851 against water temperature (°C; A) and log-initial dry weight (log(µg); B). Symbols indicate species (see inlet
852 figure); *mt* indicates muscle tissue *sRD* assays. Inserts: The same plot with symbols indicating endogenous
853 (white fill) and exogenous (black fill) life stages.

854 **Figure 7** Time to death (*Dd*) based on the percentile approach plotted against temperature (°C; A) and log-initial
855 dry weight (log(µg); B). Symbols indicate species (see inlet figure); *mt* indicates muscle tissue *sRD* assays.
856 Inserts: The same plot with symbols indicating endogenous (white fill) and exogenous (black fill) life stages.

857 **Figure 8** Time to death (*Dd*) based on the percentile approach plotted against observed time to death (*Dd*).
858 Symbols indicate species (see inlet figure); *mt* indicates muscle tissue *sRD* assays. Regression lines: y=x for
859 visual support. Left from this line, time to death is underestimated and mortality not (yet) expected. Insert A: The
860 same plot showing only endpoint trials, i.e. ending with 100 % larval mortality. Insert B: Time to death, based on
861 means (*Dd*) against observed time to death (*Dd*).

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Table 1. Overview of 15 laboratory-based food-deprivation experiments compiled in the current study. Given are the number of individual value pairs of body size and biochemical condition in each experiment (n), the number (#) of trials using different water temperatures (*T*, °C) and initial body sizes (*BS*). RNA and DNA standard curve slope ratios (dimensionless) and standardization factors SF_{pi} (dimensionless) were used for intercalibration of results (Caldarone et al., 2006) from different *RD* assays [1 – Caldarone et al. (2001), 2 – Clemmesen (1993), 3 – Suneetha et al. (1999), 4 – Westermann and Holt (1988), 5 – Wagner et al. (1998)]. Analyzed tissue types: *wb* – whole body, *mt* – muscle tissue preparations. References to each experiment are given in the last column.

Experiment ID	Species	n	# trials	# *T*	# initial *BS*	slope ratio	Standard. factor SF_{pi}	RD assay	Tissue type	Reference
A	*G. morhua*	161	5	1	5	0.81	0.34	1	*wb*	Meyer (unpublished)
B	*G. morhua*	195	5	1	5	0.75	0.31	1	*wb*	Meyer (unpublished)
C	*G. morhua*	129	1	1	1	0.79	0.33	1	*wb*	Meyer (unpublished)
D	*G. morhua*	237	3	3	1	2.60	1.08	1	*wb*	Caldarone et al. (2003)
E	*G. morhua*	80	2	1	2	1.89	0.79	1	*wb*	Meyer (unpublished)
F	*C. harengus*	875	7	7	1	2.20	0.92	2	*wb*	Harrer (2006)
G	*C. harengus*	551	12	2	12	2.20	0.92	3	*wb*	Folkvord et al. (2009)
H	*C. harengus*	135	4	1	4	2.03	0.85	2	*wb*	Clemmesen (1994)
I	*M. aeglefinus*	52	3	3	1	2.68	1.12	1	*wb*	Caldarone (2005)
J	*S. sprattus*	103	1	1	1	0.77	0.32	1	*mt*	Peck et al. (in prep)
K	*P. microps*	431	5	5	1	2.20	0.92	2	*wb*	Petereit (unpublished)
L	*C. oxyrinchus*	273	2	2	1	2.20	0.92	2	*mt*	Malzahn et al. (2003)
M	*C. albula*	213	3	3	3	2.20	0.92	2	*wb*	Petereit (unpublished)
N	*P. lethostigma*	68	1	1	1	3.90	1.63	4	*mt*	Faulk and Holt (2009)
O	*S. aurata*	39	1	1	1	5.50	2.29	5	*wb*	Faria et al. (2011)
Sum	**9**	**3542**	**55**	**33**	**40**					

Table 2. Summarized results from food deprivation trials for all species in the present study: number of individuals (n), initial age (dph), yolk sac stage (*YS*), duration of the trial (d), water temperature (*T*, °C) and initial dry weight (DW_{ini}, µg), significance value *p* of temperature effect before (*T* effect d) and after (*T* effect *Dd*) degree-day normalization and dry weight effect (*DW* effect) on starvation time [tested within chosen experiments (indicated by horizontal lines), ANCOVA, significant difference in parameter estimates compared to reference trial (*ref.*)]. Significant differences are indicated by an asterisk (*). Starvation rates (Dd^{-1}, ±SE), normalized *sRD* start value (90^{th} percentile *sRD*), *sRD* threshold (10^{th} percentile *sRD*) and time to death (*Dd*) are given for those trials where significant regression lines could be fitted to 75^{th} percentile data. *n.s.* – not significant, n.k. – not known.

Exp ID	Trial #	Species	n	Initial age (dph)	*YS*	Duration (d)	*T* (°C)	*T* effect d	*T* effect *Dd*	DW_{ini} (µg)	*DW* effect	Starvation rate (Dd^{-1})	±SE	90^{th} percentile *sRD*	10^{th} percentile *sRD*	Time to death (*Dd*)
A	1	*G. morhua*	40	8	+	5	7.0	-	-	35.9	0.001*	-0.01251	0.00344	2.6	1.2	59.1
	2	*G. morhua*	36	12	-	4	7.0	-	-	42.6	0.593	*n.s.*	-	-	-	-
	3	*G. morhua*	12	17	-	4	7.0	-	-	65.0	0.384	-0.02065	0.00154	3.0	1.5	33.4
	4	*G. morhua*	37	17	-	7	7.0	-	-	61.4	0.079	-0.01498	0.00348	3.8	1.5	60.6
	5	*G. morhua*	36	25	-	6	7.0	-	-	88.0	*ref.*	*n.s.*	-	-	-	-
B	6	*G. morhua*	42	7	+	3	13.0	-	-	43.5	0.009*	*n.s.*	-	-	-	-
	7	*G. morhua*	25	10	-	2	13.0	-	-	61.5	0.557	*n.s.*	-	-	-	-
	8	*G. morhua*	47	13	-	4	13.0	-	-	95.6	0.454	-0.01346	0.00329	3.8	1.6	62.9
	9	*G. morhua*	40	16	-	3	13.0	-	-	172.8	0.734	*n.s.*	-	-	-	-
	10	*G. morhua*	41	18	-	3	13.0	-	-	214.0	*ref.*	-0.01023	0.00077	2.7	1.8	42.9
C	11	*G. morhua*	129	9	-	3	10.0	-	-	50.9	-	*n.s.*	-	-	-	-
D	12	*G. morhua*	90	2	+	18	2.6	0.002*	0.074	71.2	-	-0.01924	0.00167	2.8	1.3	39.5
	13	*G. morhua*	97	0	+	10	5.8	0.081	0.330	70.4	-	-0.01342	0.00141	3.1	1.3	64.3
	14	*G. morhua*	50	2	+	10	9.0	*ref.*	*ref.*	71.6	-	*n.s.*	-	-	-	-
E	15	*G. morhua*	62	1	+	11	10.0	-	-	69.2	0.006*	-0.00774	0.00187	4.9	1.5	156.1
	16	*G. morhua*	19	15	-	6	10.0	-	-	107.0	*ref.*	*n.s.*	-	-	-	-

Table 2. *continued.*

Exp ID	Trial #	Species	n	Initial age (dph)	Duration (d)	*YS*	*T* (°C)	*T* effect d	*T* effect *Dd*	DW_{ini} (μg)	*DW* effect	Starvation rate (Dd^{-1})	±SE	90th percentile *sRD*	10th percentile *sRD*	Time to death (*Dd*)
F	17	*C. harengus*	125	0	12	+	3.5	0.000*	0.097	56.8	-	*n.s.*	-	-	-	-
	18	*C. harengus*	125	0	12	+	5.5	0.001*	0.111	56.8	-	-0.00299	0.00054	2.3	1.6	110.3
	19	*C. harengus*	131	0	12	+	7.5	0.004*	0.069	56.8	-	*n.s.*	-	-	-	-
	20	*C. harengus*	124	0	12	+	9.5	0.038*	0.307	56.8	-	-0.00632	0.00133	2.3	1.0	134.3
	21	*C. harengus*	121	0	12	+	11.5	0.192	0.653	56.8	-	-0.00682	0.00090	2.3	0.8	157.6
	22	*C. harengus*	124	0	12	+	13.5	0.700	0.752	56.8	-	-0.00733	0.00068	2.3	0.6	174.2
	23	*C. harengus*	125	0	12	+	15.5	*ref.*	*ref.*	56.8	-	-0.00718	0.00097	2.3	0.5	198.5
G	24	*C. harengus*	43	14	7	-	6.0	-	-	228.3	0.000*	-0.00689	0.00177	2.5	1.9	45.0
	25	*C. harengus*	49	14	7	-	6.0	-	-	288.8	0.000*	*n.s.*	-	-	-	-
	26	*C. harengus*	40	28	5	-	6.0	-	-	227.0	0.000*	*n.s.*	-	-	-	-
	27	*C. harengus*	49	28	7	-	6.0	-	-	525.2	0.173	-0.01125	0.00063	3.8	2.2	48.4
	28	*C. harengus*	49	42	7	-	6.0	-	-	344.1	0.000*	-0.00884	0.00242	2.7	1.5	66.6
	29	*C. harengus*	50	42	7	-	6.0	-	-	902.6	*ref.*	-0.01105	0.00141	4.0	2.3	48.4
	30	*C. harengus*	38	14	5	-	10.0	-	-	207.4	0.000*	*n.s.*	-	-	-	-
	31	*C. harengus*	45	14	7	-	10.0	-	-	500.2	0.104	*n.s.*	-	-	-	-
	32	*C. harengus*	39	28	5	-	10.0	-	-	213.7	0.000*	-0.00979	0.00211	2.0	1.0	74.3
	33	*C. harengus*	48	28	7	-	10.0	-	-	1739.0	0.492	-0.00687	0.00032	3.9	2.2	79.9
	34	*C. harengus*	50	42	7	-	10.0	-	-	464.4	0.001*	-0.00894	0.00170	2.6	1.2	89.6
	35	*C. harengus*	51	42	7	-	10.0	-	-	6051.1	*ref.*	-0.00725	0.00168	3.7	2.3	66.3
H	36	*C. harengus*	25	21	7	-	14.5	-	-	215.6	0.818	*n.s.*	-	-	-	-
	37	*C. harengus*	41	27	9	-	14.5	-	-	296.7	0.365	*n.s.*	-	-	-	-
	38	*C. harengus*	35	33	8	-	14.7	-	-	391.2	0.433	-0.00599	0.00076	3.8	1.5	150.3
	39	*C. harengus*	34	42	9	-	15.2	-	-	537.5	*ref.*	-0.00759	0.00158	4.7	1.3	170.0

Table 2. *continued.*

Exp ID	Trial #	Species	n	Initial age (dph)	Duration (d)	*YS*	*T* (°C)	*T* effect d	*T* effect *Dd*	DW_{ini} (μg)	*DW* effect	Starvation rate (Dd^{-1})	±SE	90th percentile *sRD*	10th percentile *sRD*	Time to death (*Dd*)
I	40	*M. aeglefinus*	18	2	16	+	5.0	0.014	0.929	699.7	-	-0.01314	0.00093	3.9	1.5	75.9
	41	*M. aeglefinus*	16	3	13	+	7.9	0.253	0.937	680.7	-	n.s.	-	-	-	-
	42	*M. aeglefinus*	18	3	11	+	10.0	*ref.*	*ref.*	746.7	-	n.s.	-	-	-	-
J	43	*S. sprattus*	103	*n.k.*	12	-	18.0	-	-	43230.0	-	-0.00428	0.00132	4.8	1.3	305.3
K	44	*P. microps*	92	*n.k.*	12	-	9.8	0.000	0.005*	556.3	-	-0.00573	0.00088	2.4	1.0	146.4
	45	*P. microps*	92	*n.k.*	12	-	13.0	0.000	0.066	556.3	-	-0.00726	0.00080	2.4	0.8	151.5
	46	*P. microps*	89	*n.k.*	12	-	16.2	0.011	0.358	556.3	-	-0.00632	0.00087	2.4	0.6	229.4
	47	*P. microps*	84	*n.k.*	11	-	19.2	0.240	0.903	556.3	-	-0.00640	0.00090	2.4	0.5	239.6
	48	*P. microps*	74	*n.k.*	9	-	24.1	*ref.*	*ref.*	556.3	-	-0.00746	0.00069	2.5	0.5	223.9
L	49	*C. oxyrinchus*	189	1	36	+	8.4	0.070	0.993	1291.0	-	-0.00447	0.00064	1.6	0.3	409.7
	50	*C. oxyrinchus*	84	1	16	+	17.4	*ref.*	*ref.*	1291.0	-	n.s.	-	-	-	-
M	51	*C. albula*	59	17	12	+	3.7	-	-	291.2	-	n.s.	-	-	-	-
	52	*C. albula*	55	5	12	+	7.4	0.022*	0.162	388.3	-	-0.00480	0.00080	1.2	0.6	124.7
	53	*C. albula*	115	5	24	+	8.5	*ref.*	*ref.*	454.4	-	-0.00481	0.00039	1.0	0.4	192.8
N	54	*P. lethostigma*	68	51	10	-	18.3	-	-	18865.3	-	-0.00500	0.00033	4.8	1.6	224.8
O	55	*S. aurata*	39	35	3	-	21.6	-	-	2144.4	-	n.s.	-	-	-	-

Figure 1.

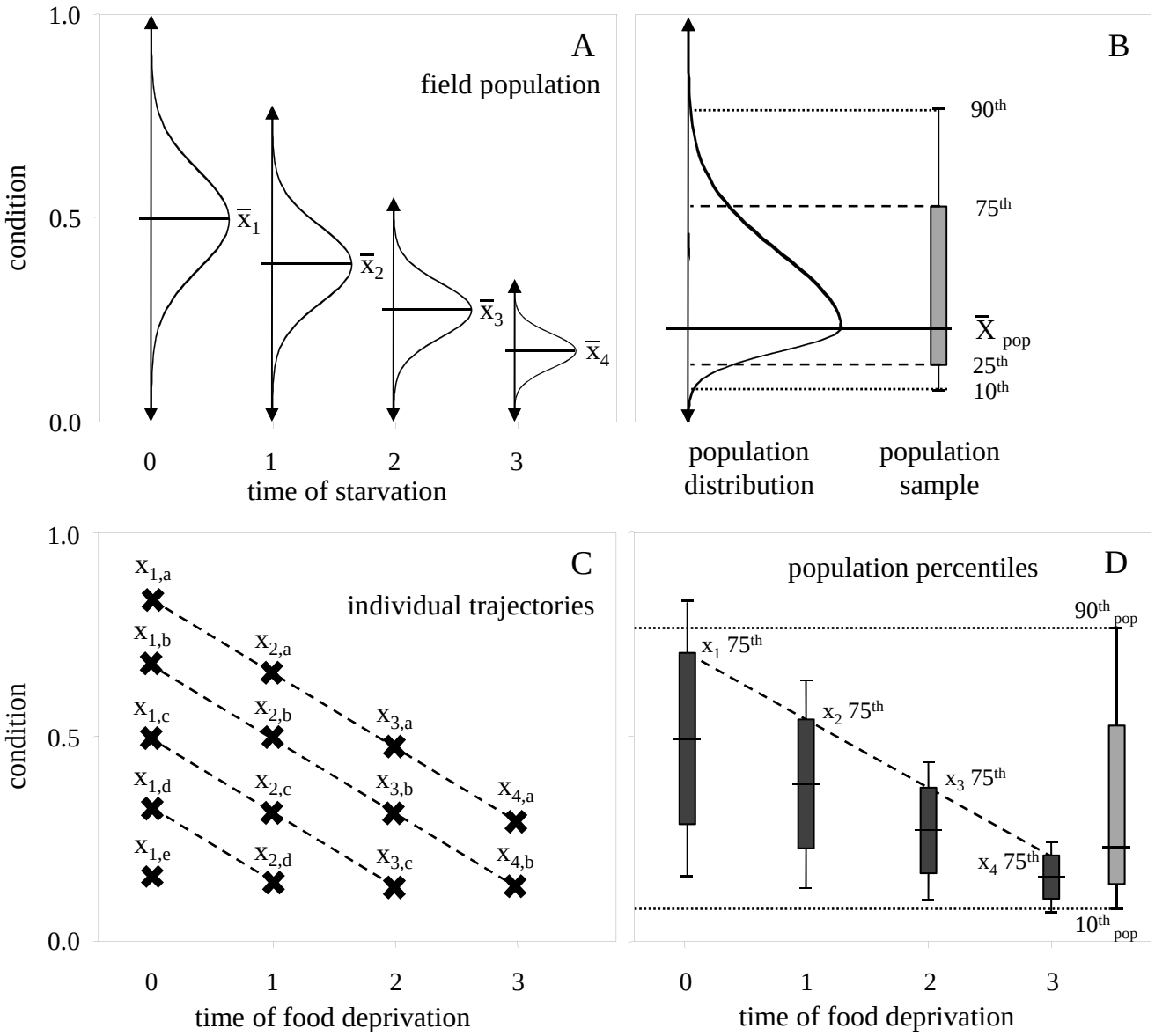

A
field population
B
C
individual trajectories
D
population percentiles
condition
time of starvation
time of food deprivation
population distribution
population sample
90th
75th
25th
10th
90th pop
10th pop

Figure 2.

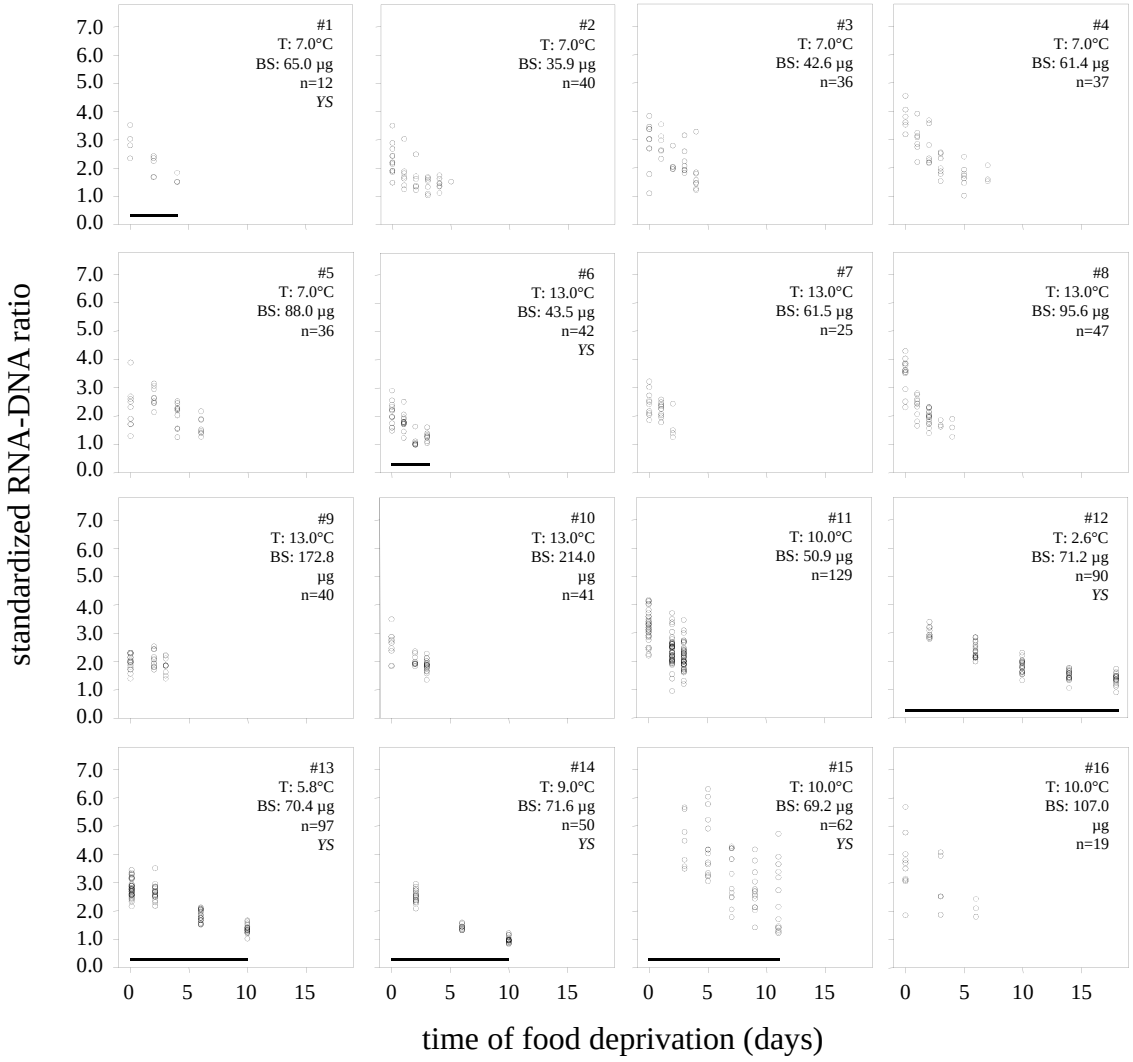

standardized RNA-DNA ratio
time of food deprivation (days)
#1
T: 7.0°C
BS: 65.0 µg
n=12
YS
#2
T: 7.0°C
BS: 35.9 µg
n=40
#3
T: 7.0°C
BS: 42.6 µg
n=36
#4
T: 7.0°C
BS: 61.4 µg
n=37
#5
T: 7.0°C
BS: 88.0 µg
n=36
#6
T: 13.0°C
BS: 43.5 µg
n=42
YS
#7
T: 13.0°C
BS: 61.5 µg
n=25
#8
T: 13.0°C
BS: 95.6 µg
n=47
#9
T: 13.0°C
BS: 172.8 µg
n=40
#10
T: 13.0°C
BS: 214.0 µg
n=41
#11
T: 10.0°C
BS: 50.9 µg
n=129
#12
T: 2.6°C
BS: 71.2 µg
n=90
YS
#13
T: 5.8°C
BS: 70.4 µg
n=97
YS
#14
T: 9.0°C
BS: 71.6 µg
n=50
YS
#15
T: 10.0°C
BS: 69.2 µg
n=62
YS
#16
T: 10.0°C
BS: 107.0 µg
n=19

Figure 3.

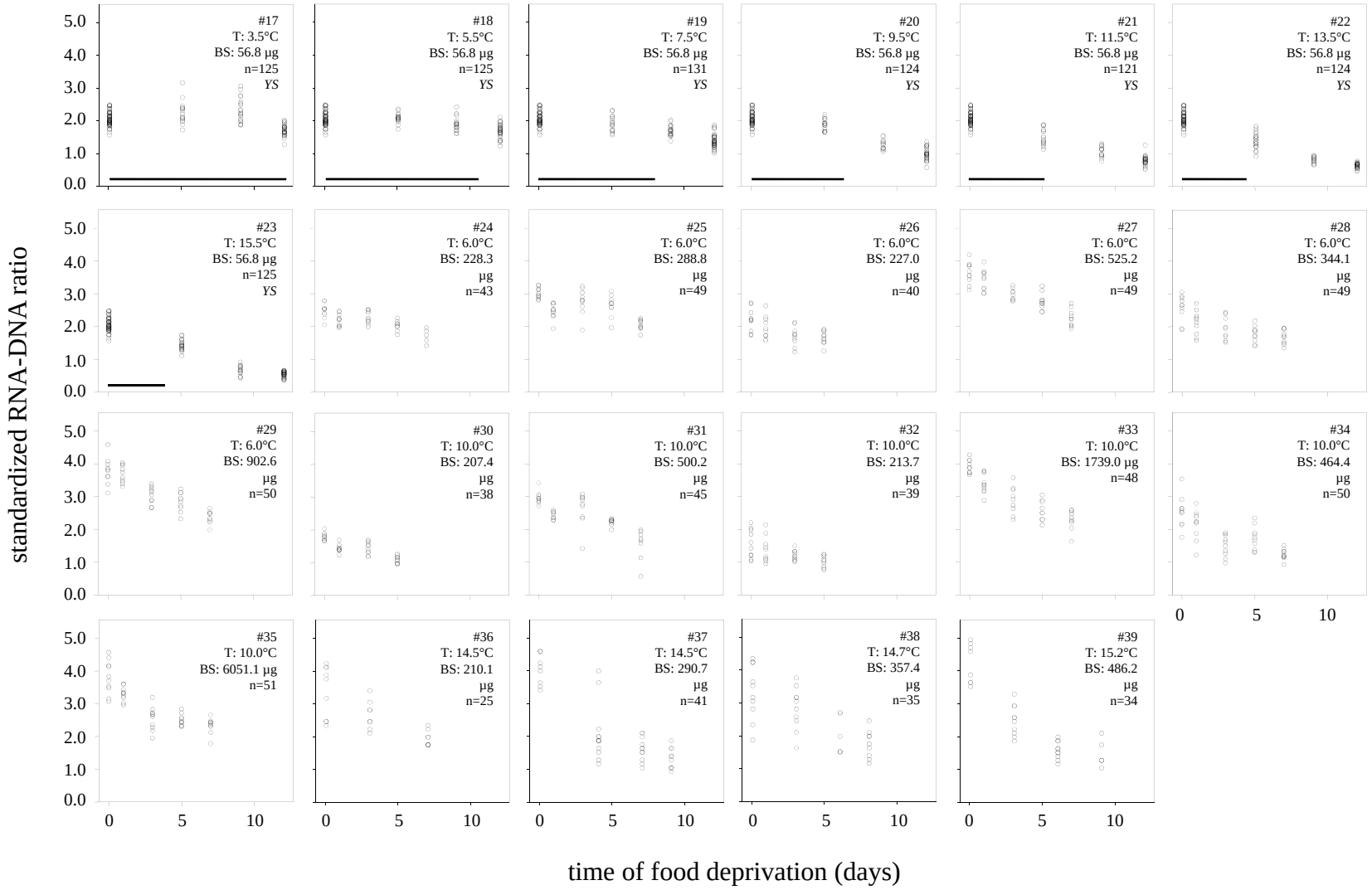

standardized RNA-DNA ratio
time of food deprivation (days)
#17
T: 3.5°C
BS: 56.8 µg
n=125
YS
#18
T: 5.5°C
BS: 56.8 µg
n=125
YS
#19
T: 7.5°C
BS: 56.8 µg
n=131
YS
#20
T: 9.5°C
BS: 56.8 µg
n=124
YS
#21
T: 11.5°C
BS: 56.8 µg
n=121
YS
#22
T: 13.5°C
BS: 56.8 µg
n=124
YS
#23
T: 15.5°C
BS: 56.8 µg
n=125
YS
#24
T: 6.0°C
BS: 228.3 µg
n=43
#25
T: 6.0°C
BS: 288.8 µg
n=49
#26
T: 6.0°C
BS: 227.0 µg
n=40
#27
T: 6.0°C
BS: 525.2 µg
n=49
#28
T: 6.0°C
BS: 344.1 µg
n=49
#29
T: 6.0°C
BS: 902.6 µg
n=50
#30
T: 10.0°C
BS: 207.4 µg
n=38
#31
T: 10.0°C
BS: 500.2 µg
n=45
#32
T: 10.0°C
BS: 213.7 µg
n=39
#33
T: 10.0°C
BS: 1739.0 µg
n=48
#34
T: 10.0°C
BS: 464.4 µg
n=50
#35
T: 10.0°C
BS: 6051.1 µg
n=51
#36
T: 14.5°C
BS: 210.1 µg
n=25
#37
T: 14.5°C
BS: 290.7 µg
n=41
#38
T: 14.7°C
BS: 357.4 µg
n=35
#39
T: 15.2°C
BS: 486.2 µg
n=34

Figure 4.

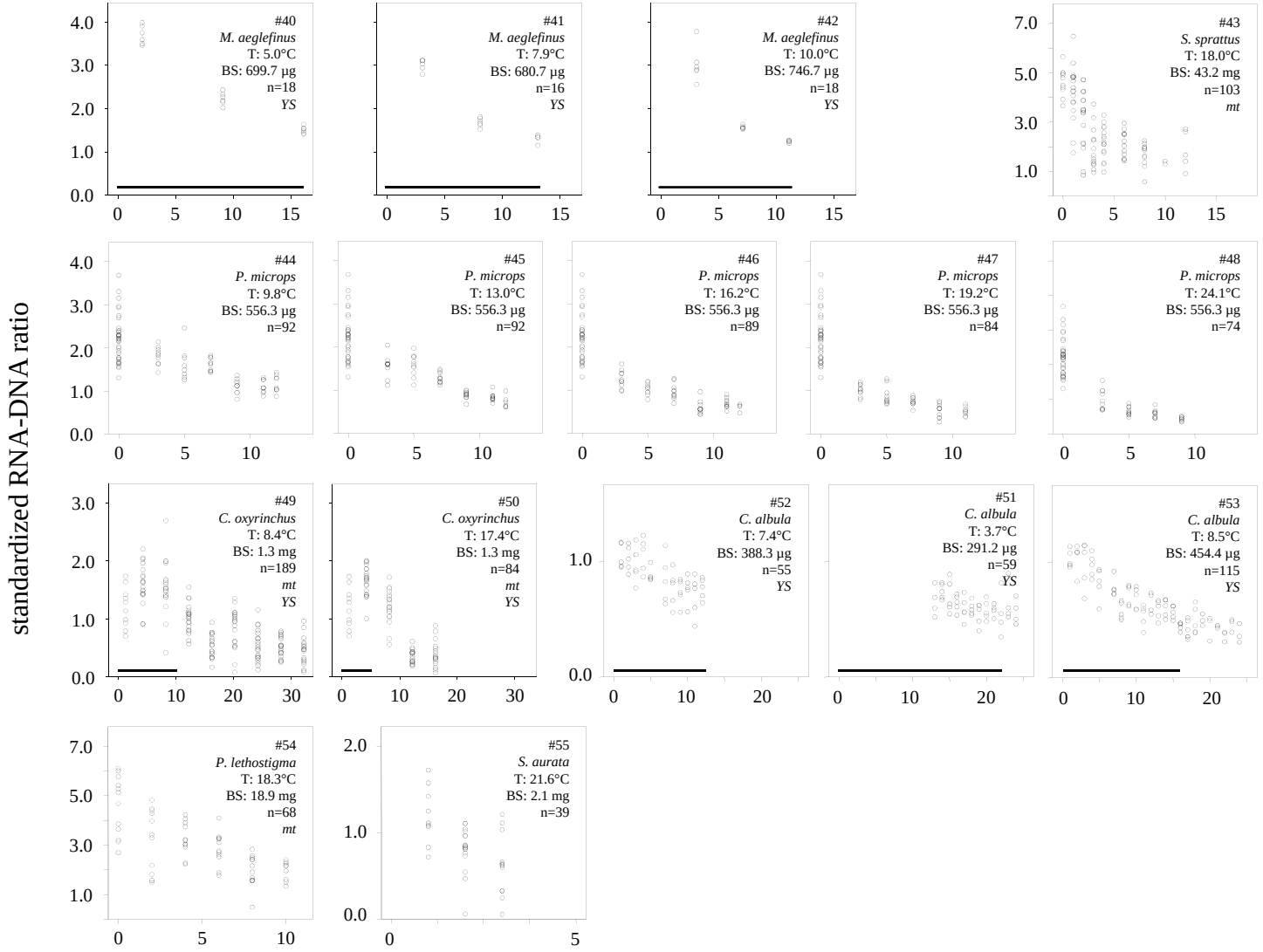

standardized RNA-DNA ratio
#40
M. aeglefinus
T: 5.0°C
BS: 699.7 µg
n=18
YS
#41
M. aeglefinus
T: 7.9°C
BS: 680.7 µg
n=16
YS
#42
M. aeglefinus
T: 10.0°C
BS: 746.7 µg
n=18
YS
#43
S. sprattus
T: 18.0°C
BS: 43.2 mg
n=103
mt
#44
P. microps
T: 9.8°C
BS: 556.3 µg
n=92
#45
P. microps
T: 13.0°C
BS: 556.3 µg
n=92
#46
P. microps
T: 16.2°C
BS: 556.3 µg
n=89
#47
P. microps
T: 19.2°C
BS: 556.3 µg
n=84
#48
P. microps
T: 24.1°C
BS: 556.3 µg
n=74
#49
C. oxyrinchus
T: 8.4°C
BS: 1.3 mg
n=189
mt
YS
#50
C. oxyrinchus
T: 17.4°C
BS: 1.3 mg
n=84
mt
YS
#52
C. albula
T: 7.4°C
BS: 388.3 µg
n=55
YS
#51
C. albula
T: 3.7°C
BS: 291.2 µg
n=59
YS
#53
C. albula
T: 8.5°C
BS: 454.4 µg
n=115
YS
#54
P. lethostigma
T: 18.3°C
BS: 18.9 mg
n=68
mt
#55
S. aurata
T: 21.6°C
BS: 2.1 mg
n=39
time of food deprivation (days)

Figure 5.

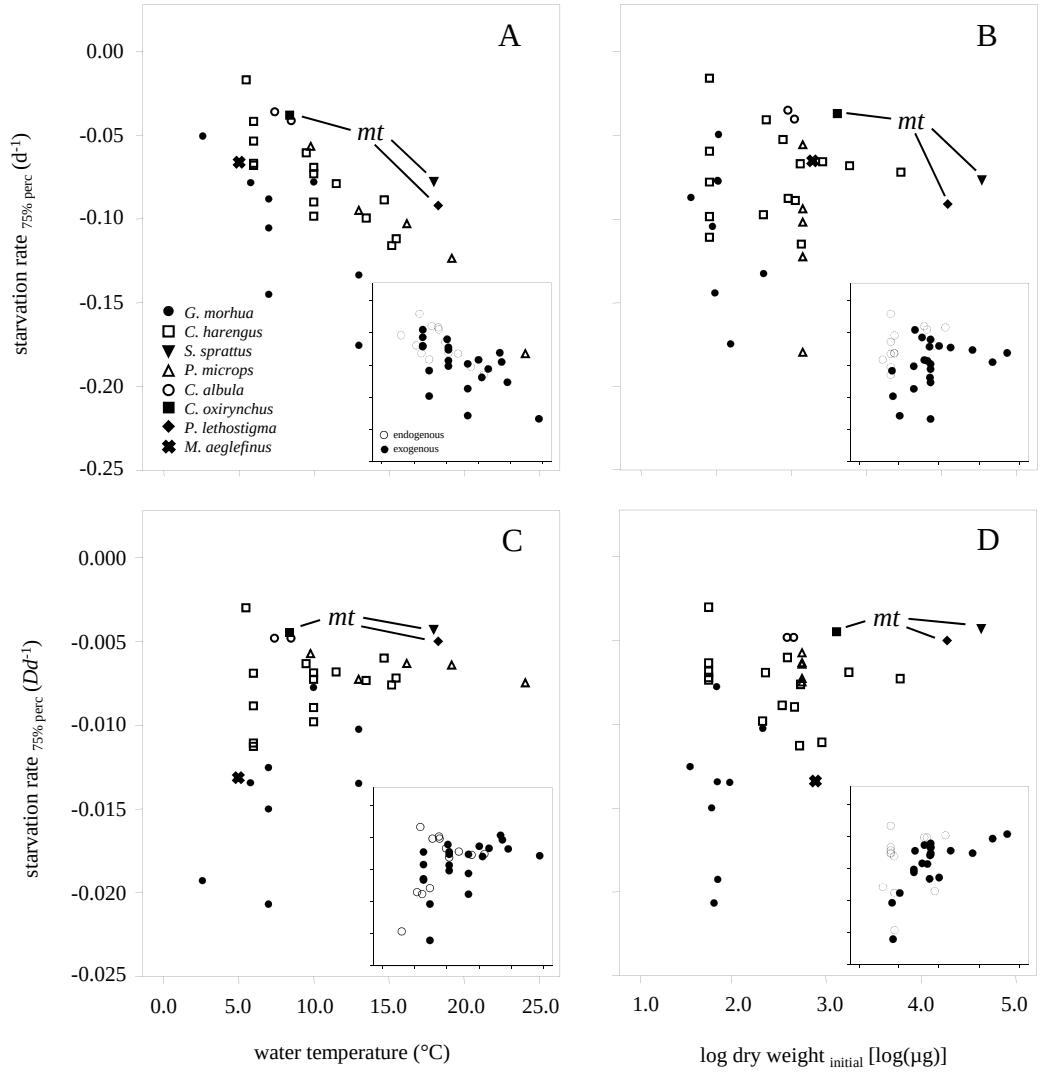

A
B
C
D
mt
starvation rate 75% perc (d-1)
starvation rate 75% perc (Dd-1)
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
0.000
-0.005
-0.010
-0.015
-0.020
-0.025
G. morhua
C. harengus
S. sprattus
P. microps
C. albula
C. oxirynchus
P. lethostigma
M. aeglefinus
endogenous
exogenous
0.0
5.0
10.0
15.0
20.0
25.0
water temperature (°C)
1.0
2.0
3.0
4.0
5.0
log dry weight initial [log(µg)]

Figure 6.

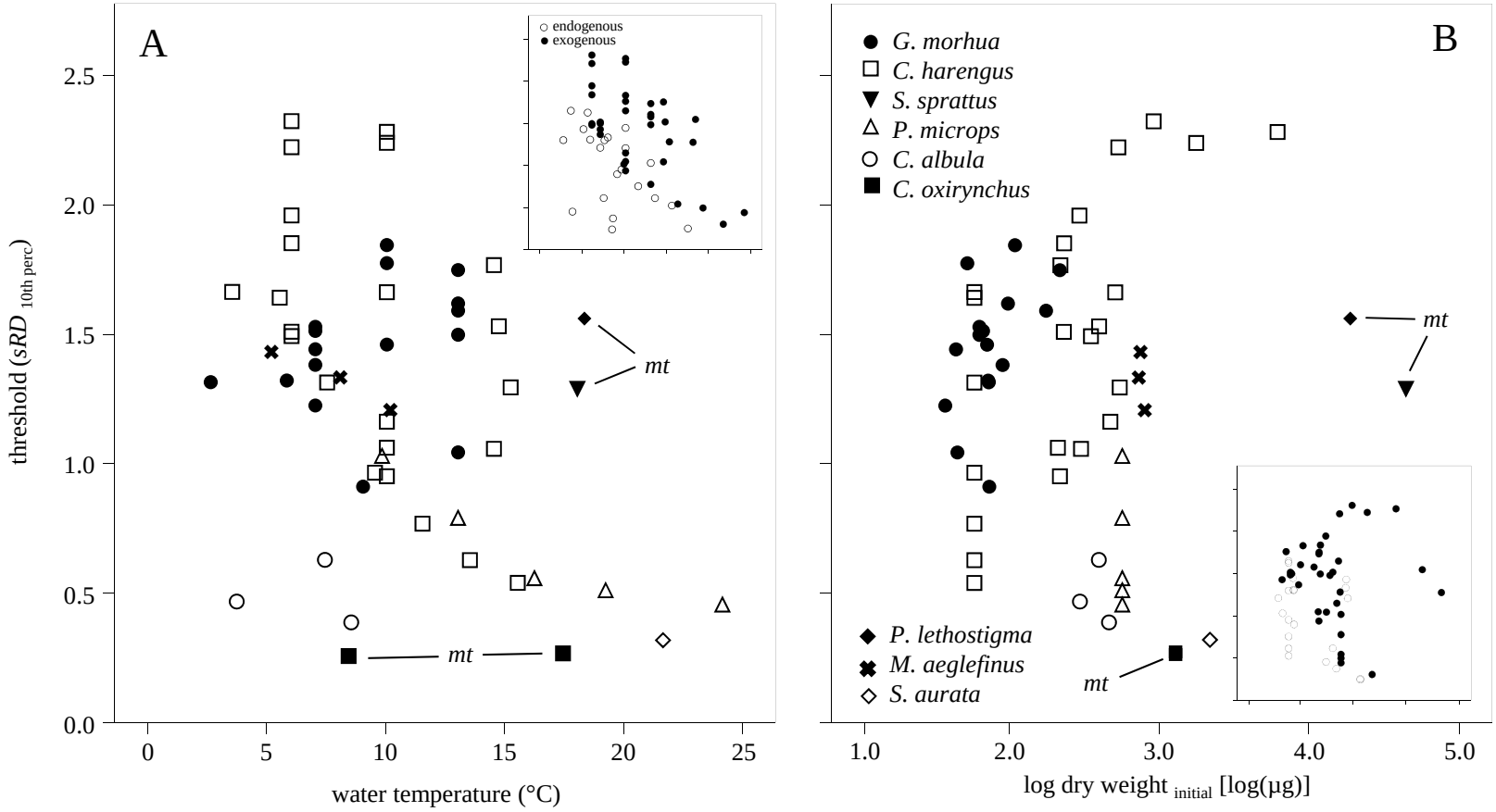

A
B
endogenous
exogenous
mt
mt
mt
G. morhua
C. harengus
S. sprattus
P. microps
C. albula
C. oxirynchus
P. lethostigma
M. aeglefinus
S. aurata
threshold (sRD 10th perc)
water temperature (°C)
log dry weight initial [log(µg)]
0.0
0.5
1.0
1.5
2.0
2.5
0
5
10
15
20
25
1.0
2.0
3.0
4.0
5.0

Figure 7.

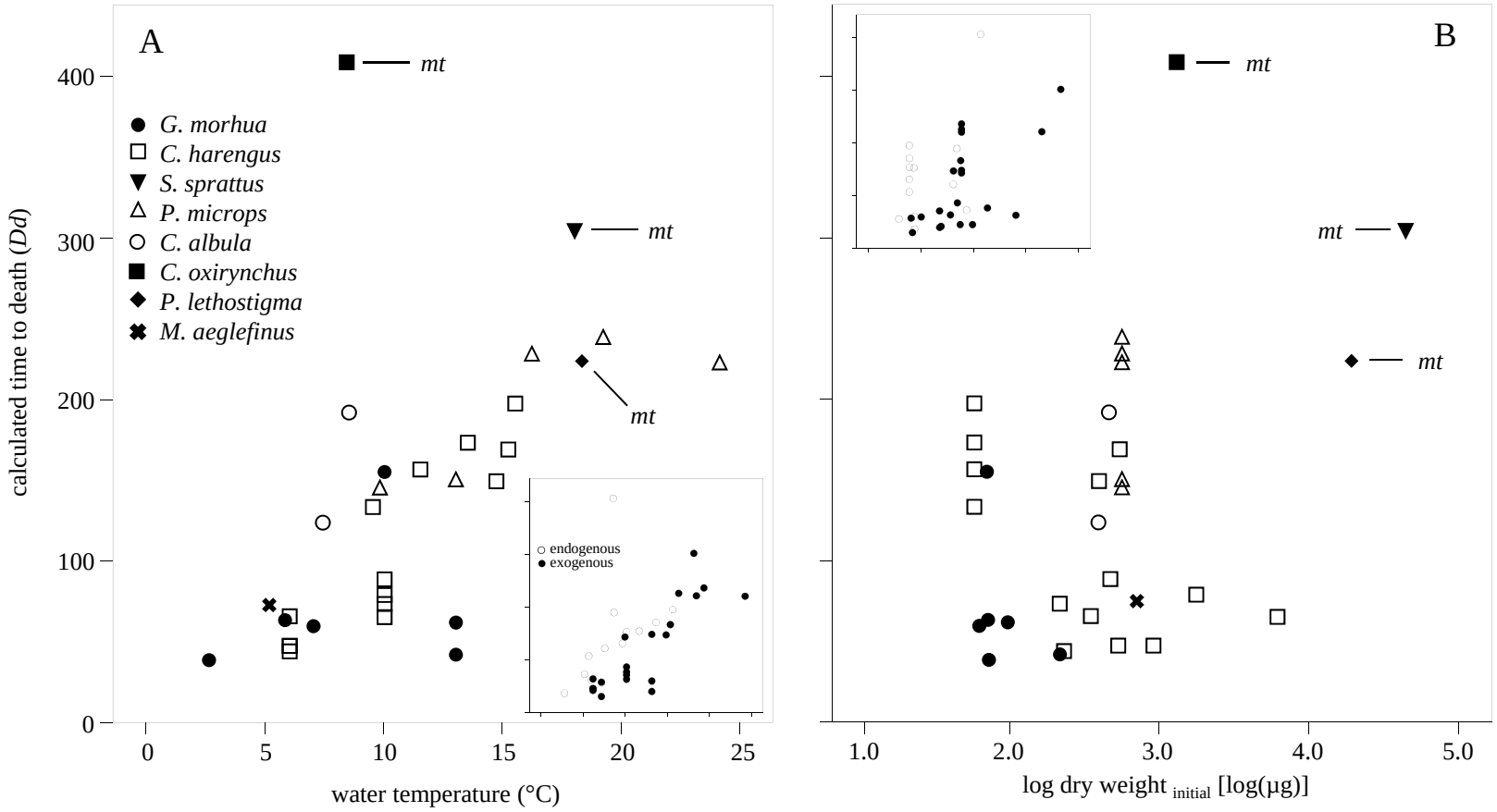
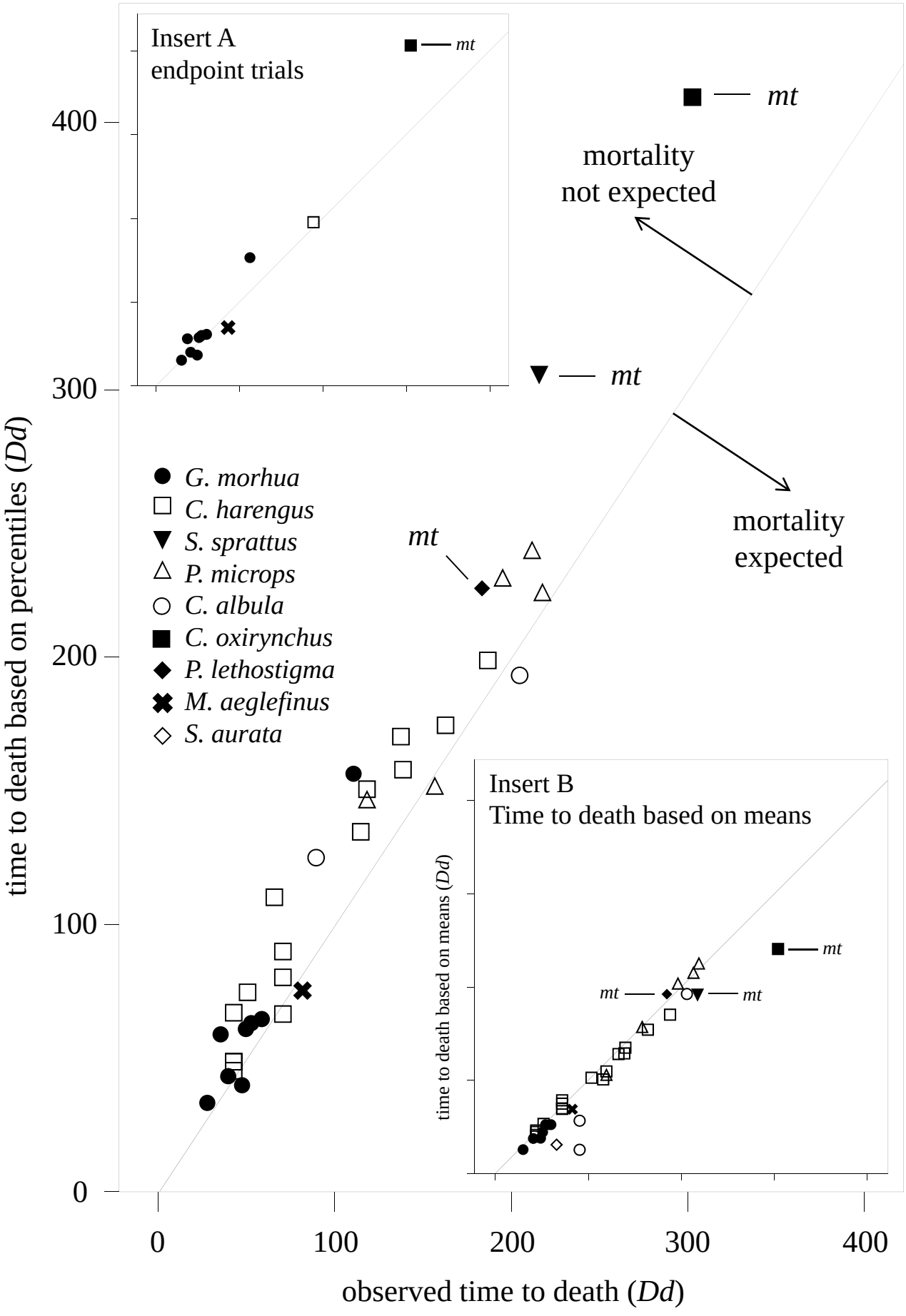

A
B
mt
G. morhua
C. harengus
S. sprattus
P. microps
C. albula
C. oxirynchus
P. lethostigma
M. aeglefinus
endogenous
exogenous
calculated time to death (Dd)
water temperature (°C)
log dry weight initial [log(µg)]
0
100
200
300
400
0
5
10
15
20
25
1.0
2.0
3.0
4.0
5.0

Figure 8.


Insert A
endpoint trials
mt
mt
mortality
not expected
mt
mortality
expected
mt
G. morhua
C. harengus
S. sprattus
P. microps
C. albula
C. oxirynchus
P. lethostigma
M. aeglefinus
S. aurata
Insert B
Time to death based on means
time to death based on means (Dd)
mt
mt
mt
time to death based on percentiles (Dd)
observed time to death (Dd)
0
100
200
300
400