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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 fish early life stages can survive without feeding and how starvation rate and time until death are influenced by body size, temperature and species is critical to understanding processes controlling mortality in the sea. The present study is an across-species analysis of starvation-induced changes in biochemical condition in early life stages of nine marine and freshwater fishes. 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 yolk-sac and 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 75th percentiles of sampled populations versus time (degree-days, *Dd*). The 10th percentile of *sRD* successfully approximated the lowest, life-stage-specific biochemical condition (the edge of death). Temperature 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, interspecific functions predicting the relationship between growth rate and *sRD* in feeding fish larvae do not 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 of individual fish pre-recruits in the laboratory and provide useful metrics for research on the role of starvation in the sea.

1. Introduction

The recruitment (year class) strength of marine fish species can vary by orders of magnitude between years 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 when larvae are particularly susceptible to predation mortality (Bailey and Houde, 1989). Depending upon the degree of mismatch with prey production and availability, food-deprived larvae may die of starvation or weakened larvae may be more vulnerable to predation (Skajaa et al., 2004). It is therefore critical to not only assess the degree of food limitation in the sea, but to also understand how the physiological process of starvation changes with species and/or life stage to gain a mechanistic understanding of the role that prey deprivation plays in the recruitment process.

The nutritional condition of marine fish early life stages has been evaluated using the ratio of nucleic acids (RNA–DNA ratio, *RD*) for more than two decades (Buckley, 1984; Buckley et al., 2008). RNAs are essential for the biosynthesis of proteins and can vary depending on nutritional condition, 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

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(Caldarone et al., 2006) has allowed multi-species comparisons of protein-specific growth rates and *RD* in marine fish larvae resulting in a general model relating growth rate and *RD* to temperature (Buckley et al., 2008). During food deprivation, declines in *RD* have been observed in larvae of Atlantic cod (*Gadus morhua*) and Atlantic herring (*Clupea harengus*), reflecting the cessation of protein synthesis and somatic growth (e.g., Clemmesen, 1994; Grønkjær et al., 1997; Suneetha et al., 1999). Still, *RD* is not usually considered an indicator of 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, sometime leading to ambiguous patterns in changes in *RD*. Direct methods to describe and quantify the phenomenon of starvation have included observations on feeding success (gut content) (Bochdansky et al., 2008), measurements of otolith microstructure (Johnson et al., 2002), and histological examination of digestive tissues (Kjørsvik et al., 1991; Theilacker and Watanabe, 1989). However, the potential influences of life stage and/or species relationships on nutritional deficiency, *RD*, and starvation in marine fish early life stages are not yet clarified.

Laboratory trials have contributed a wealth of knowledge on 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., 2004a) or genetic differences among individuals (Case et al., 2006; Clemmesen et al., 2003; Saborido-Rey et al., 2003), and can 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 our knowledge, this aspect of starvation response was 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; b) the underlying function describing decline in *RD* with time of food deprivation is reasonably known (i.e. an exponential function); and c) larvae with a low nutritional condition suffer higher mortality rates than those in good condition (if starvation is the only source of mortality). Computing daily changes in the 75th percentile of *RD* values appears to be a good approach to estimate starvation rate since individuals within upper percentiles represent a discrete sub-group of the population that is unlikely to shift in its relative ranking within a group in the short-term (Folkvord et al., 2009; Paulsen et al., 2009). Larvae in the upper percentiles (i.e., 75th and above) will tend to survive for the longest times and will thus form an ever-increasing portion of the population on subsequent sampling days (Fig. 1), yielding a better approximation of the "true" starvation rate. The 90th and 10th percentiles of the sampling population can be categorized as idealized start and end points, representing initial condition at onset of food deprivation and a final condition near starvation-induced mortality, respectively. In our review and synthesis of *RD* research, 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 traditional approach that utilizes group mean values.

Although there is individual variability in starvation rates, ambient temperature and body size will have profound effects on 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 proposed. 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 ($T \times RD$). Folkvord (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 normalizes the contribution of temperature and body size 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 in *RD* 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 of larval fishes in the sea.

2. Material and methods

2.1. Data set overview

We compiled previously published and unpublished data from laboratory-based, food deprivation experiments conducted on nine species of marine and freshwater fish 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 experiments are described in the original publications (Table 1). The common feature of all experiments was that groups of well nourished (either newly-hatched yolk-sac or previously *ad libitum* fed) fish larvae were deprived of food for at least three days and sampled (minimum of 5 fish per sample) at the start and on at least two more occasions during the experiment.

The combined data included measurements on 3542 individuals. Paired values of body size and RNA–DNA ratio (*RD*) were acquired from 15 experiments (Table 1). Experiments included 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 (mean initial dry weight, *DW*) ranged from 35.9 μg in young larvae to 43.2 mg in juveniles. Endogenous feeding yolk-sac larvae were included in 21 trials. Trial duration ranged from 2 to 36 days (Table 2). The termination of each trial did not necessarily coincide with fish mortality. To be included in our analysis, sampled larvae from an experiment must have been processed using only one technique for measurement of body size and one single-dye fluorescence-based protocol to determine bulk nucleic acid contents. If not stated otherwise, *DW* was measured after freeze-drying to the nearest 0.1 μg (for <100 μg individuals) or 1.0 μg (for >100 μg individuals). In some cases, *DW* was calculated from known relationships to standard length [Experiment H, herring, Harrer (2006); Experiment N, southern flounder, Bolasina et al. (2006) and Qin et al. (2008)], to protein content (Experiment D, cod and Experiment I, haddock, Caldarone, unpublished data), or to wet weight (Experiment J, sprat, Peck et al., 2004b).

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,

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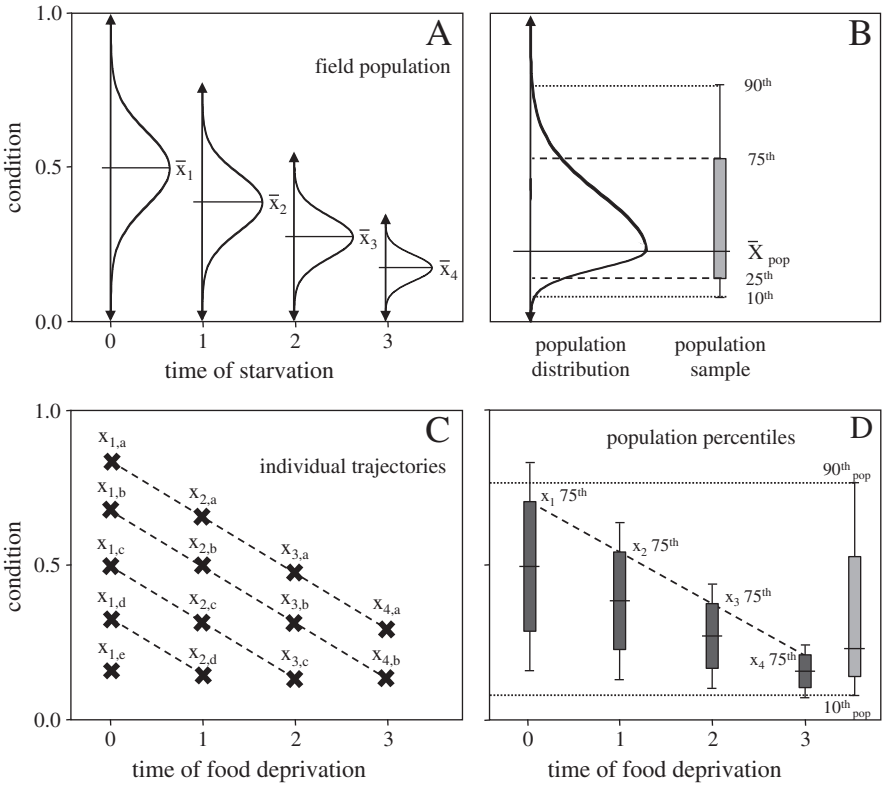

Fig. 1. Conceptual illustration of the percentile approach. Panel A: Field populations contain at any time individuals under food deprivation. Condition is a function of time of starvation and is stochastically distributed around unknown mean values for each time-step of starvation. B: Condition in field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

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 concentrations. 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*) are referred to throughout the remainder of this manuscript. Assay-specific slope ratios and standardization factors are provided (Table 1).

Measurements of *sRD* were conducted 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

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					

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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}, ±S.E.), normalized *sRD* start value (90th percentile *sRD*), *sRD* threshold (10th percentile *sRD*) and time to death (*Dd*) are given for those trials where significant regression lines could be fitted to 75th 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})	±S.E.	90th percentile *zsRD*	10th percentile *sRD*	Time to death (*Dd*)
A	1	*G. morhua*	40	8	+	5	7.0	–	–	35.9	<0.01*	−0.01251	0.00344	2.6	2.6	59.1
	2	*G. morhua*	36	12	–	4	7.0	–	–	42.6	0.59	*n.s.*	–	–	–	–
	3	*G. morhua*	12	17	–	4	7.0	–	–	65.0	0.38	−0.02065	0.00154	3.0	1.5	33.4
	4	*G. morhua*	37	17	–	7	7.0	–	–	61.4	0.08	−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	<0.01*	*n.s.*	–	–	–	–
	7	*G. morhua*	25	10	–	2	13.0	–	–	61.5	0.56	*n.s.*	–	–	–	–
	8	*G. morhua*	47	13	–	4	13.0	–	–	95.6	0.45	−0.01346	0.00329	3.8	1.6	62.9
	9	*G. morhua*	40	16	–	3	13.0	–	–	172.8	172.8	*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	<0.01*	0.07	0.07	–	−0.01924	0.00167	2.8	1.3	39.5
	13	*G. morhua*	97	0	+	10	5.8	0.08	0.33	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	<0.01*	−0.00774	0.00187	4.9	1.5	156.1
	16	*G. morhua*	19	15	–	6	10.0	–	–	107.0	*ref.*	*n.s.*	–	–	–	–
F	17	*C. harengus*	125	0	12	+	3.5	<0.01*	0.10	56.8	–	*n.s.*	–	–	–	–
	18	*C. harengus*	125	0	12	+	5.5	<0.01*	0.11	56.8	–	−0.00299	0.00054	2.3	1.6	110.3
	19	*C. harengus*	131	0	12	+	7.5	<0.01*	0.07	56.8	–	*n.s.*	–	–	–	–
	20	*C. harengus*	124	0	12	+	9.5	<0.05*	0.31	56.8	–	−0.00632	0.00133	2.3	1.0	134.3
	21	*C. harengus*	121	0	12	+	11.5	0.19	0.65	56.8	–	−0.00682	0.00090	2.3	0.8	157.6
	22	*C. harengus*	124	0	12	+	13.5	0.70	0.75	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	<0.01*	−0.00689	0.00177	2.5	1.9	45.0
	25	*C. harengus*	49	14	7	–	6.0	–	–	288.8	<0.01*	*n.s.*	–	–	–	–
	26	*C. harengus*	40	28	5	–	6.0	–	–	227.0	<0.01*	*n.s.*	–	–	–	–
	27	*C. harengus*	49	28	7	–	6.0	–	–	525.2	0.17	−0.01125	0.00063	3.8	2.2	48.4
	28	*C. harengus*	49	42	7	–	6.0	–	–	344.1	<0.01*	−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	<0.01*	*n.s.*	–	–	–	–
	31	*C. harengus*	45	14	7	–	10.0	–	–	500.2	0.10	*n.s.*	–	–	–	–
	32	*C. harengus*	39	28	5	–	10.0	–	–	213.7	<0.01*	−0.00979	0.00211	2.0	1.0	74.3
	33	*C. harengus*	48	28	7	–	10.0	–	–	1739.0	0.49	−0.00687	0.00032	3.9	2.2	79.9
	34	*C. harengus*	50	42	7	–	10.0	–	–	464.4	<0.01*	−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	0.82	*n.s.*	–	–	–	–
	37	*C. harengus*	41	27	9	–	14.5	–	–	296.7	0.37	*n.s.*	–	–	–	–
	38	*C. harengus*	35	33	8	–	14.7	–	–	391.2	0.43	−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
I	40	*M. aeglefinus*	18	2	16	+	5.0	<0.05*	0.93	699.7	–	−0.01314	0.00093	3.9	1.5	75.9
	41	*M. aeglefinus*	16	3	13	+	7.9	0.25	0.94	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	<0.01*	<0.01*	556.3	–	−0.00573	0.00088	2.4	1.0	146.4
	45	*P. microps*	92	*n.k.*	12	–	13.0	<0.01*	0.07	556.3	–	−0.00726	0.00080	2.4	0.8	151.5
	46	*P. microps*	89	*n.k.*	12	–	16.2	<0.05*	0.36	556.3	–	−0.00632	0.00087	2.4	0.6	229.4
	47	*P. microps*	84	*n.k.*	11	–	19.2	0.24	0.90	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	0.07	0.99	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	<0.05*	0.16	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	51	–	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.	–	–	–	–

the unpredictable effects of differences in dissection protocols and potential differences in cell size (Olivar et al., 2009). However, muscle tissue *sRD* of early life stages was previously demonstrated to be affected by food deprivation and it does respond to the physiological process of starvation. Sample tissue types are therefore indicated throughout the manuscript and tissue types were treated separately in all analysis. We followed a similar approach for endogenous feeding, yolk-sac larvae (Table 2). The contribution of maternal RNA and yolk-sac dry weight substantially affect *sRD* and estimates of body size, and can introduce a bias to modeled growth estimates (Buckley et al., 2006). Nevertheless, yolk-sac larval starvation parameters 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 from regression models fit to the 75th percentile of each sampling

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date *sRD* regressed on 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 as a linear relationship, values of *sRD* were natural logarithm- ($\log_e$) transformed. If not stated otherwise, all analyses utilized $\log_e$-transformed 75th percentile *sRD* data. Only starvation rate parameters (rate of decrease and time to death) from significant linear regressions ($p \leq 0.05$) were included in further analysis.

2.4. Temperature-normalization of starvation rate

A subset of data was used to validate the degree-day normalization of starvation rates (Experiment D, #12-14; F, #17-23; I, #40-42; K, #44-48; L, #49 + 50; M, #52 + 53; Table 2). Only trials with different rearing temperatures in 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 analysis of covariance (ANCOVA) to test for significant differences between starvation rates at different temperatures (independent variable). The ANCOVA was also conducted with temperature-normalized data, expressing time of food deprivation in degree-days (*Dd*). Temperature-normalization was considered effective when a significant effect of temperature (the independent variable) in the original data became insignificant after normalization.

2.5. Body size and life stage effect on starvation rate

A subset of data was analyzed 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; Table 2). Only trials with different initial dry weight in the same experiment were compared to minimize uncontrolled effects (e.g., maternal/batch effects, species differences). Yolk-sac and exogenous feeding larvae were compared in three larval cod experiments (Experiments A, B and E). Only one experiment (Experiment G, herring) spanned a sufficient body-size range to compare early-stage, exogenous feeding larvae with older-stage larvae. Time of food deprivation, expressed in degree-days (*Dd*), was the covariate and mean initial dry weight the independent variable in an ANCOVA.

2.6. Population percentiles and time to death

Time to death was calculated as:

$$time\ to\ death = \frac{log_e\left(sRD_{10th\ perc}\right) - log_e\left(sRD_{90th\ perc}\right)}{starvation\ rate_{75th\ perc}} \quad (1)$$

where $\log_e(sRD_{10\text{th perc}})$ and $\log_e(sRD_{90\text{th perc}})$ represent the 10th and 90th percentile values of $\log_e$-transformed *sRD* for the sampled population and $starvation\ rate_{75\text{th perc}}$ represents the temperature-normalized starvation rate based on the 75th percentile of daily $\log_e$-transformed *sRD* (Fig. 1). The 10th percentile of *sRD* values in any population of food-deprived individuals that expresses substantial variability in condition is presumed to approximate the lowest level of biochemical condition sustaining life. A direct estimate for this threshold level was difficult to derive from the present dataset because most of the food deprivation trials ended before larval mortality (33 out of 55 trials), and the 75th percentile values of *sRD* calculated on the last sampling day of these trials may not represent larvae ultimately close to death. Therefore, we assumed that the 10th percentile of the full sampled population (pooled over time) was a better approximation for the edge of death. The 90th percentile is thought to represent larvae having the highest species- and life stage-specific *sRD* and thus provides a normalized start-value for the onset of food deprivation.

2.7. Validation of the percentile approach

To evaluate 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. In the subset of trials that ended with larval mortality, two *sRD*-based estimates of the 'time to death', one calculated using percentile data (Eq. (1)) and the other using average starting and final *sRD* values ("mean-based"), were regressed against the observed time to death (i.e., trial duration). Only experimental trials yielding a significant slope parameter estimate (for each of the respective regressions) were included in this regression analysis.

In trials using whole body tissue preparations, the 10th percentile *sRD* 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 evaluate this metric for fish larvae and juveniles under food depletion. The model parameters of their equation no. 1 (Buckley et al., 2008), including an interaction term between *sRD* and T, were used for the calculation.

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). Standardized regression coefficient ß was reported for multiple regressions. 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*) and muscle tissue (*mt*) preparations, and in yolk-sac and exogenous-feeding life stages. Examples of these trends for cod, herring and other species are provided (Figs. 2–4). Starvation rates, i.e. significant linear regressions of the 75th percentile of $\log_e$-transformed *sRD* versus time (*Dd*) of food deprivation, were calculated for 34 trials and ranged from −0.0206 $*Dd^{-1}$ in 17 days post hatch (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 slower than the mean (−0.0091 $*Dd^{-1}$) whole body starvation rate (Mann–Whitney test, U = 5.0, z = −2.52, $p < 0.05$). When considering only whole-body preparations, starvation rates of yolk-sac larvae (−0.0089 ± 0.0047 $*Dd^{-1}$) did not differ significantly from exogenous-feeding larvae rates (−0.0093 ± 0.0038 $*Dd^{-1}$, Mann–Whitney test, U = 97.0, z = −0.689, p > 0.05). Starvation rates (mean ± S.D.; no S.D. indicated for $n = 1$ observations) for taxa in the analysis, ranked in descending order, are *G. morhua* −0.0140(0.0043) > *M. aeglefinus* −0.0131 > grand mean −0.0087(0.0041) > *C. harengus* −0.0077(0.0021) > *P. microps* −0.0066(0.0007) > *P. lethostigma* −0.0050 > *C. albula* −0.0048(0.0001) > *C. oxyrinchus* −0.0045 > *S. sprattus* −0.0043 $*Dd^{-1}$. Trials in which the regression slope was not significant (21 trials) included occurrences for all species, temperatures and body sizes (indicating no bias).

3.1. Temperature-normalization of starvation rate

Temperature had a significant influence on $\log_e$-transformed 75th percentile *sRD* before temperature-normalization, i.e. when time of food deprivation was expressed in days (Table 2). Significant differences (ANCOVA) relative to the reference trial (highest temperature in the same experiment) were detected in every experiment in the subset of data, except for Experiment L (*C. oxyrinchus*, yolk-sac

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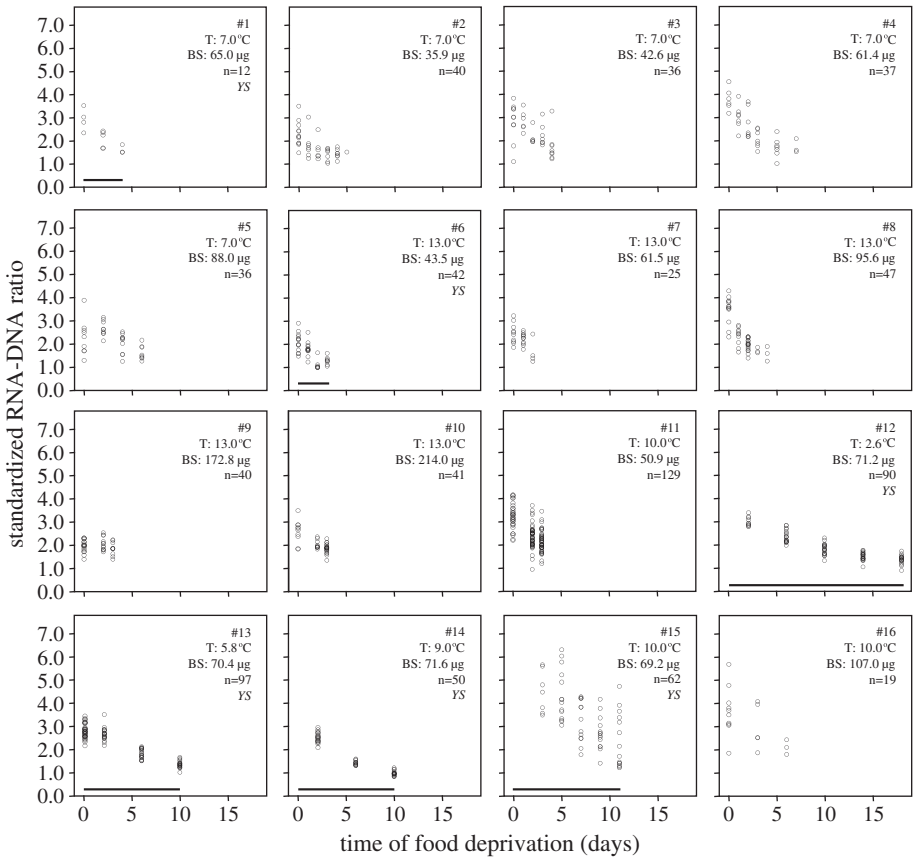

Fig. 2. Untransformed data overview for cod (*Gadus morhua*) food deprivation trials. Standardized RNA–DNA ratio of individuals (y axis) plotted against time of food deprivation (days; x axis). Black bars indicate the presence of endogenous yolk reserves. Samples were analyzed as whole body homogenates. T – temperature (°C), BS – body size/dry weight (µg), YS – yolk-sac stage. Panels are arranged by trial (#) numbers (Table 2).

larvae). After degree-day normalization, no significant differences relative to the reference trial were detected, indicating that the time of food-depletion (x-axis) was successfully normalized so that differences in the decrease of *sRD* (y-axis) over time became insignificant. The only exception was one trial at the lowest temperature of Experiment K (*P. microps*, exogenous feeding larvae). This experiment spanned the broadest temperature range (14.3 °C) for a single species in the entire dataset. A failing of the *T*-normalization was observed between the two extreme ends of the *T*-range in this experiment, indicating a possible limitation in general applicability of this normalization procedure when applied over such a broad temperature range.

Temperature explained a significant proportion of variability in the starvation rates of the full dataset before *Dd*-normalization (linear regression, $B = -0.0056$ (0.0011), $p < 0.001$, $r^2 = 0.48$; Fig. 5A). The slope of the regression indicated that faster starvation rates were found at higher water temperatures. After *Dd*-normalization, the slope of the regression was still negative but it was reduced by more than one order of magnitude (and not significantly different from zero), indicating that the *T*-effect was successfully channeled into *Dd* ($B = -0.0004$ (0.0001), $p = 0.027$, $r^2 = 0.16$; Fig. 5C).

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 yolk-sac and exogenous-feeding larvae were equally included in the analysis (Experiments A, B and E). In these three experiments, starvation rates of yolk-sac larvae were significantly different (ANCOVA, Table 2) from the reference category. The reference category had the highest initial dry weight, whereas the yolk-sac larvae had the lowest initial *DW* in the respective experiment. When yolk-sac larvae were excluded, no significant difference was found. From the two experiments that did not contain any yolk-sac 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 is apparent that at approximately 500 µg dry weight there is a break point for detecting body size differences in starvation rate. At sizes smaller than 500 µg dry weight (Experiment H) and heavier than 500 µg dry weight (Experiment G) body size had no effect on starvation rate. Exploratory analysis of starvation rates with respect to body size (here: $\log_{10}$ dry weight) indicated a significant correlation between starvation rate and body size in *Dd*-normalized starvation rates ($B = -0.002$ (0.001), $p = 0.014$, $r^2 = 0.18$; Fig. 5D), but not in the original data ($B = -0.005$ (0.009), $p = 0.591$, $r^2 = 0.01$; Fig. 5B). Examining starvation rates only in those experimental trials that used exogenous feeding larvae and whole body tissue preparations, increased the proportion of explained variability in starvation rate from 18 to 45% ($B = -0.005$ (0.001), $p = 0.002$, $r^2 = 0.45$; Fig. 5D).

3.3. Population percentiles and time to death

The value of *sRD* at the 10th percentile, 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 also was variable across temperature (Fig. 6A) and body size (Fig. 6B). It was significantly correlated with temperature when yolk-sac ($B = -0.074$ (0.028), $p = 0.023$, $r^2 = 0.42$) and exogenous-feeding stages ($B = -0.084$ (0.021), $p = 0.001$, $r^2 = 0.48$) were analyzed separately. The slopes of these regressions were nearly identical, but the intercept for exogenous-feeding larvae was higher (2.4 ± 0.3, estimator ± standard error, S.E.) than for yolk-sac larvae (1.7 ± 0.3). There was no significant correlation with body size ($B = 0.260$ (0.307), $p = 0.409$, $r^2 = 0.04$), but life-stage specific trends for cod and

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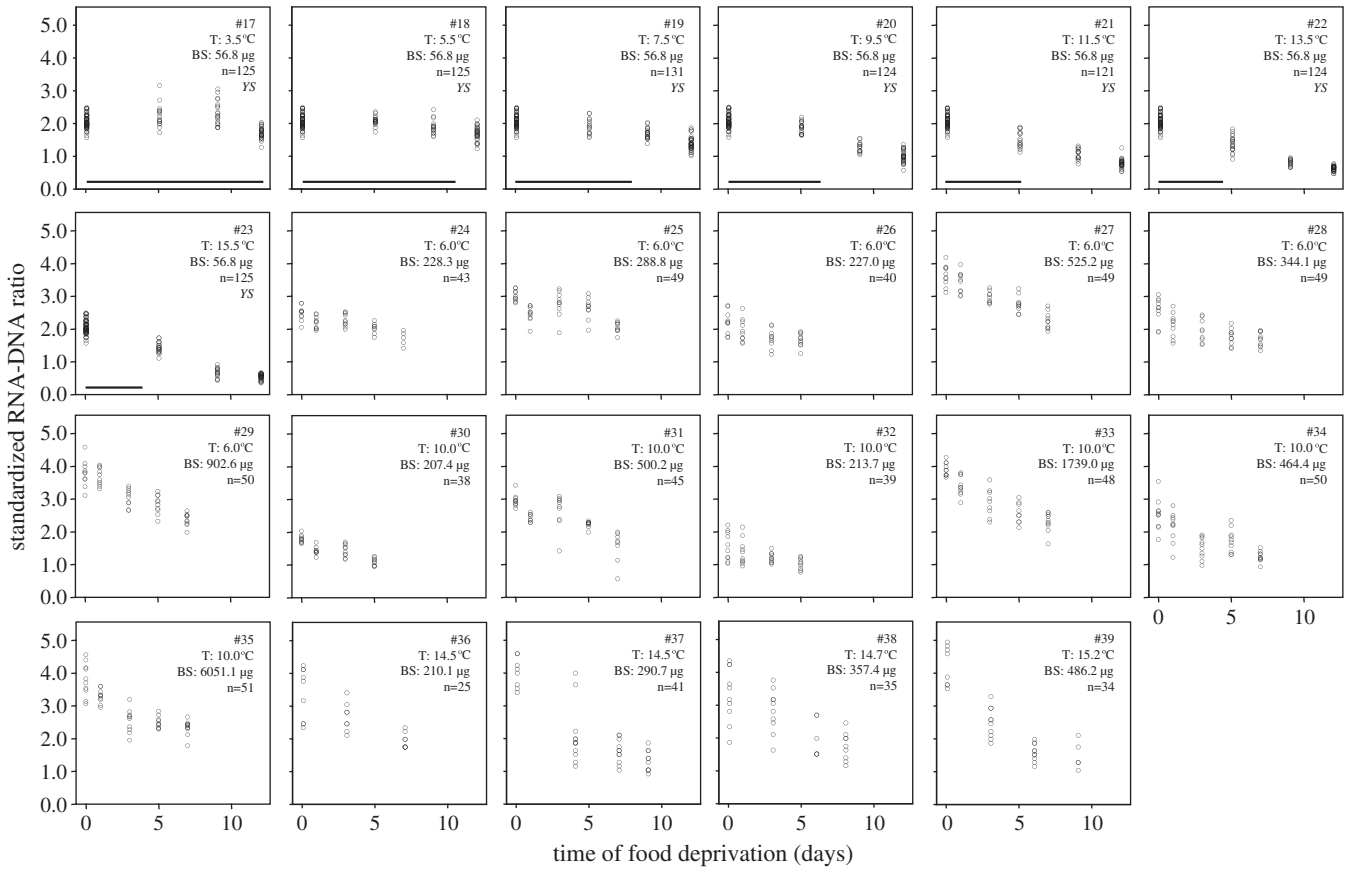

Fig. 3. Untransformed data overview for herring (*Clupea harengus*) food deprivation trials. Standardized RNA–DNA ratio of individuals (y axis) plotted against time of food deprivation (days; x axis). Black bars indicate the presence of endogenous yolk reserves. Samples were analyzed as whole body homogenates. T – temperature (°C), BS – body size/dry weight (µg), YS – yolk-sac stage. Panels are arranged by trial (#) numbers (Table 2).

herring larvae were apparent. On average, yolk-sac larvae of these two species had lower 10th percentile values (1.1 ± 0.1, mean ± S.E.) than exogenous-feeding stages (1.7 ± 0.1), whereas life-stage-independent mean values for the 10th percentile *sRD* were very similar (1.5 ± 0.04 and 1.5 ± 0.2). The 90th percentile, the approximation for *sRD* at onset of food deprivation, ranged from 1.0 to 4.9 across species and life stages and was not correlated with 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 with temperature in both yolk-sac and exogenous-feeding stages (Fig. 7A), but not with body size (Fig. 7B). A multiple regression using temperature and body size (*DW*) as predictors explained 59% of the observed variability, but *DW* alone was not significant (Temperature: B = 10.284 (1.632), ß = 0.76, $p<0.001$, change in $r^2 = 0.57$; dry weight: $p = 0.59$). The slopes of the regressions between time to death and temperature in yolk-sac larvae ($B = 11.719$ (1.797), ß = 0.85, $p<0.001$, $r^2 = 0.71$) and exogenous-feeding larvae ($B = 12.396$ (2.420), ß = 0.85, $p<0.001$, $r^2 = 0.72$) were both positive. Time to death increased by about 12 *Dd* with each degree rise in temperature for the two life stages.

3.4. Validation of the percentile approach

Starvation rates based on the 75th percentile *sRD* were regressed against starvation rates calculated from the arithmetic mean *sRD* of daily sampled populations ($B = 0.997$ (0.044), $p<0.001$, $r^2 = 0.95$). The slope of the regression did not differ significantly from 1 (Confidence Interval (CI) range for slope estimator $B = 0.907$ to 1.088). The 10th percentile *sRD* (edge of death) for trials ending with larval mortality (22 trials, including yolk-sac and exogenous life stages, all tissue types) slightly overestimated the observed mean *sRD* on the last sampling day, but was nevertheless significantly correlated with it ($B = 1.123$ (0.100), $p<0.001$, $r^2 = 0.86$). The slope of this regression did not differ significantly from 1 (CI range for slope estimator $B = 0.914$ to 1.332). The 90th percentile *sRD* (normalized start-value) of all trials (except for two outliers, both Experiment E) slightly underestimated the observed mean *sRD* at onset of food deprivation ($B = 0.982$ (0.026), $p<0.001$, $r^2 = 0.96$). The slope of this regression did not differ significantly from 1 (CI range for slope estimator $B = 0.929$ to 1.035).

Calculated time to death was regressed against observed time to death (i.e., trial duration), including only experimental trials ending with larval mortality. The regression slope was significantly lower than 1 ($B = 0.738$ (0.041), $p<0.001$, $r^2 = 0.97$; CI range for slope estimator $B = 0.645$ to 0.831), indicating an underestimation of trial duration by the percentile-based parameters. Visual inspection of residuals showed that the regression was influenced by a single trial (#49, *C. oxirynchus*, *mt*, yolk-sac stage). When this trial was excluded from the analysis, the regression slope was no longer different from one ($B = 1.092$ (0.108), $p<0.001$, $r^2 = 0.93$; CI range for slope estimator $B = 0.844$ to 1.340). Time to death, calculated using both the percentile approach and the arithmetic mean, was regressed against trial duration (Fig. 8). The percentile approach significantly underestimated trial duration ($B = 0.793$ (0.035), $p<0.001$, $r^2 = 0.94$; CI range for slope estimator $B = 0.721$ to 0.865), whereas the slope of the population mean based estimator was not significantly different from 1 ($B = 1.002$ (0.044), $p<0.001$, $r^2 = 0.94$; CI range for slope estimator $B = 0.912$ to 1.091). Residuals from a one-to-one line representing 100% congruence of the calculated and the observed metric were positive in only 5 out of 34 cases, supporting the assertion that the larvae

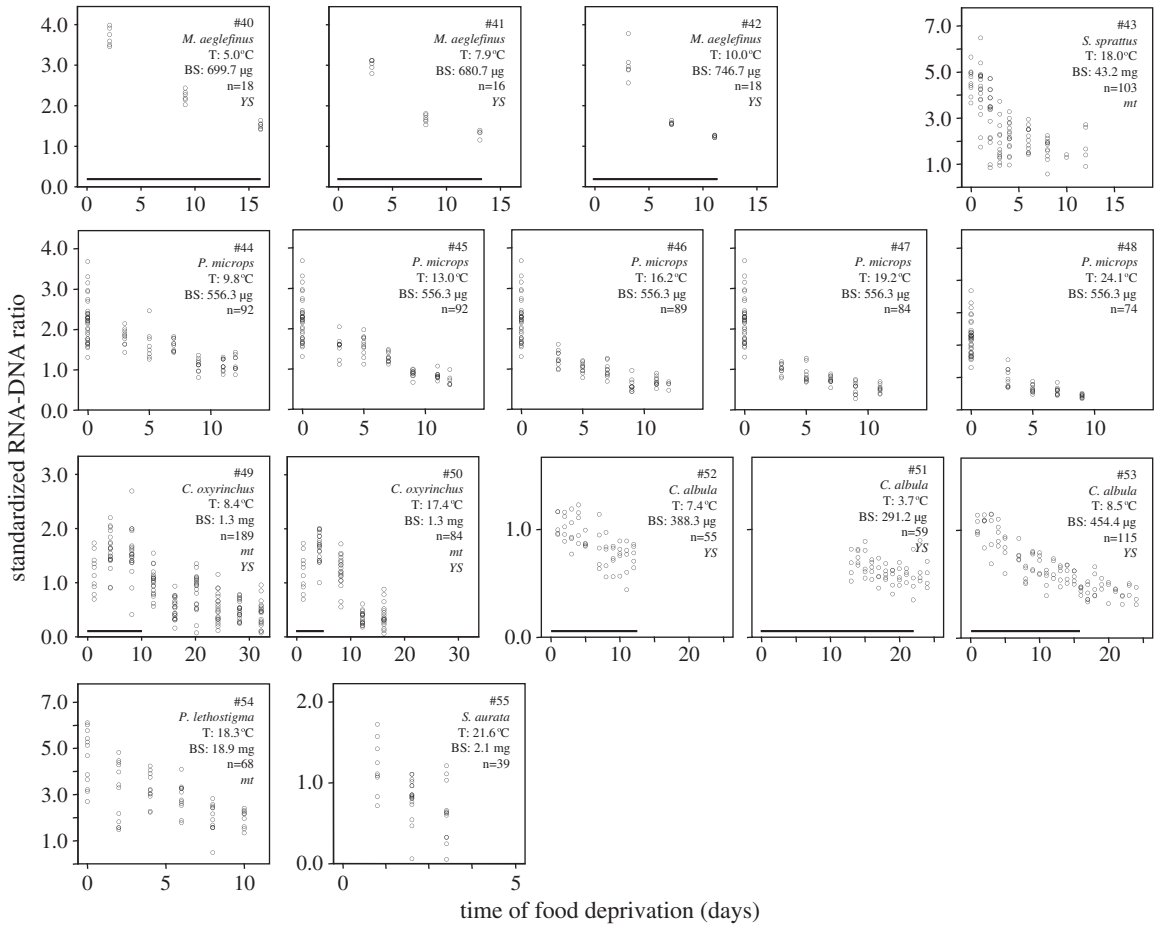

Fig. 4. Untransformed data overview for food deprivation trials in seven species. Standardized RNA–DNA ratio of individuals (y axis) plotted against time of food deprivation (days; x axis). Black bars indicate the presence of endogenous yolk reserves. Samples were either analyzed as muscle tissue (indicated,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

in most of the trials were sampled slightly before they were ultimately close to death. In contrast, in 17 out of 36 cases the population mean-based metric had negative residuals, indicating that predicted time to death was longer than that observed in the trial.

Instantaneous growth rates, calculated from 10th percentiles of whole body *sRD* using a multi-species, temperature corrected model describing instantaneous growth rates as a function of *sRD* (Buckley et al., 2008), ranged from −0.065 to 0.071 $*d^{-1}$ over all food deprivation trials. In yolk-sac stages, most of the calculated growth rates were negative (−0.026 on average, range from −0.063 to 0.008 $*d^{-1}$). In exogenous-feeding larvae, the mean 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 numerous factors acting on all life stages. The fast growth and high mortality rates observed for larval fish have led researchers to conclude that processes acting during the larval stage have the potential to introduce major variability in recruitment levels of marine fishes. The ability to gain robust in situ growth estimates and distinguish individuals that are growing well from those growing poorly is critical (Bochdansky et al., 2008; Houde, 2008). Starvation and predation are considered to be the most important causes of mortality in the early life stages of fish (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 (Bochdansky et al., 2008; Fuiman and Cowan, 2003).

Biochemical condition (*RD*) is one of the most widely used growth indicators for marine fish early life stages (e.g., Buckley, 1984; Buckley et al., 2008; Chícharo and Chícharo, 2008; Clemmesen et al., 2003). Recent research has standardized this ratio (*sRD*) allowing comparison of measurements made in different laboratories (Caldarone et al., 2006). In our study, we used a novel approach to examine changes in *sRD* in food-deprived individuals to 1) identify species- and body size-specific s*RD* lower threshold values, 2) quantify the rate of change in s*RD* in food-deprived individuals, and 3) estimate the time required to reach threshold *sRD* levels in the early life stages of nine marine and freshwater fish species. These parameters are cornerstones of theories describing mortality via starvation in finfish pre-recruit life stages (Ferron and Leggett, 1994). Additionally, we demonstrated how inter-individual variability in s*RD* can be harnessed to provide better estimates of starvation trajectories using a percentile approach. We argue that the physiological rate of starvation is better estimated using this approach compared to traditional calculations that employ group mean values.

The low *sRD* values of food-deprived individuals in the present study yielded both negative as well as positive growth rates when applying the relationship described by Buckley et al. (2008). Our results suggest that this published general model (Buckley et al., 2008) and species-specific models (e.g., Caldarone, 2005; Caldarone et al.,

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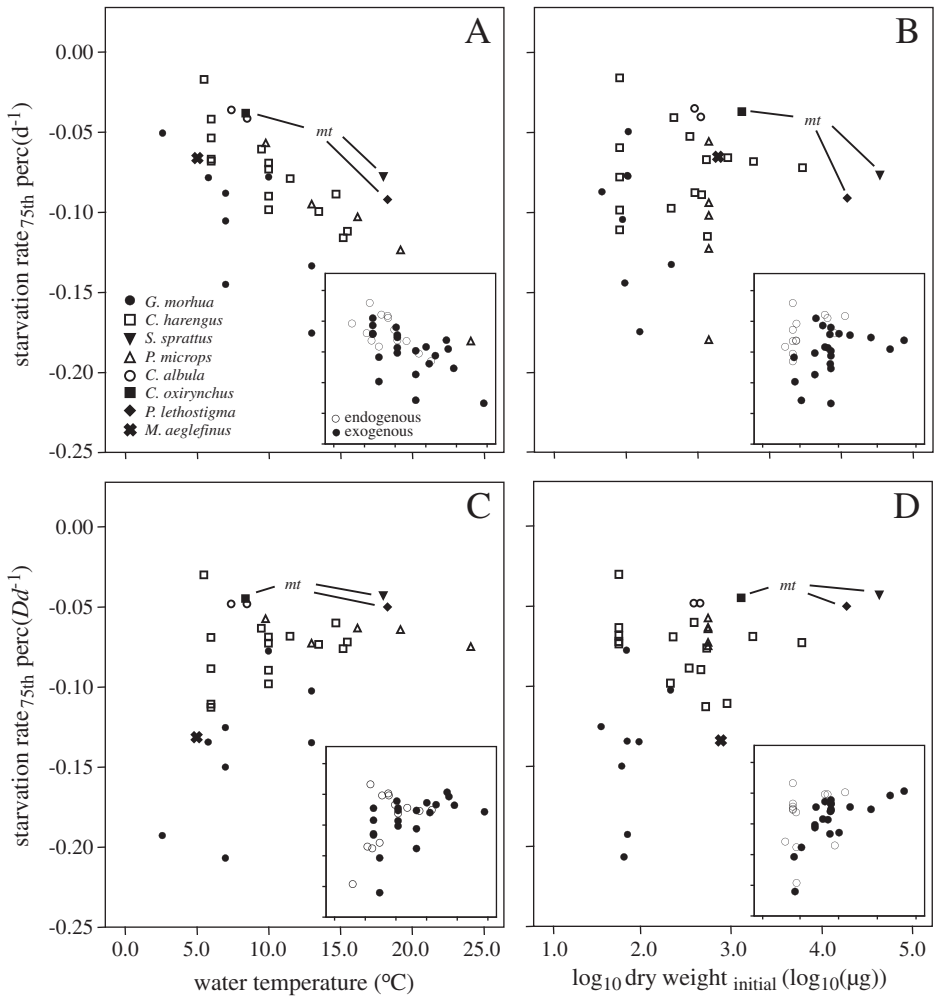

Fig. 5. Starvation rate (significant linear slope of 75th percentile $\log_e$-transformed *sRD* against time of food deprivation) before (d^{-1}; Panels A and B) and after (Dd^{-1}; Panels C and D) degree-day-transformation (y axis) plotted against water temperature (°C; x axis; Panels A and C) and $\log_{10}$-initial dry weight ($\log_{10}$ (µg); x axis; Panels B and D). Symbols indicate species (see inlet figure); *mt* indicates muscle tissue *sRD* assays. Inserts: The same plots (same x- and y-axis) depicting endogenous yolk-sac (white fill) and exogenous (black fill) life stages.

2003) explaining the relationship between growth rate and *sRD* in feeding fish larvae do not apply to individuals experiencing prolonged food deprivation. In well-nourished individuals, growth potential can be well explained by a generic (inter-specific) relationship including *sRD* values and water temperature (Buckley et al., 2008). However, this generic relationship breaks down during food

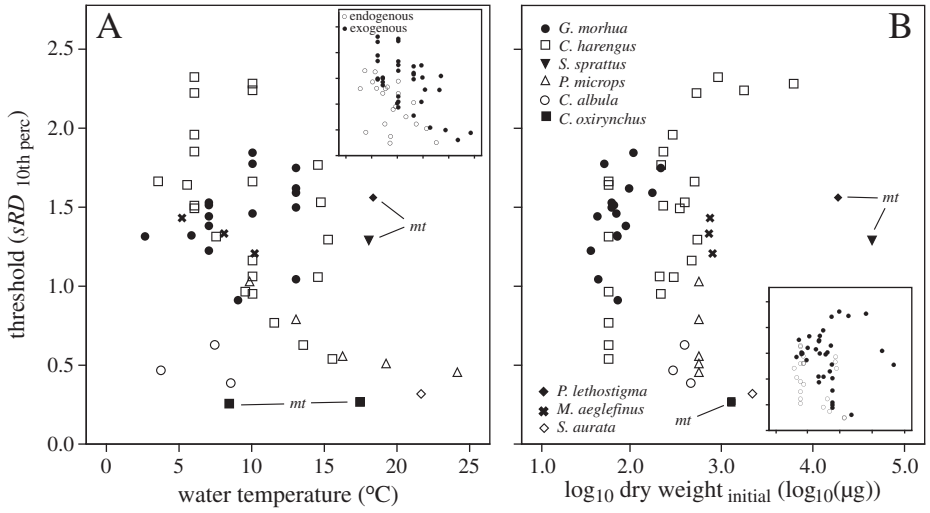

Fig. 6. Threshold *sRD* for each trial (y axis) plotted against water temperature (°C; x axis; Panel A) and $\log_{10}$-initial dry weight ($\log_{10}$(µg); x axis; Panel B). Threshold *sRD*. 10th percentile values of *sRD* are assumed to represent the lowest possible biochemical condition sustaining life and therefore the edge of death. Symbols indicate species (see figure legend); *mt* indicates muscle tissue *sRD* assays. Inserts: The same plots (same x- and y-axis) with symbols indicating endogenous yolk-sac (white fill) and exogenous (black fill) life stages.

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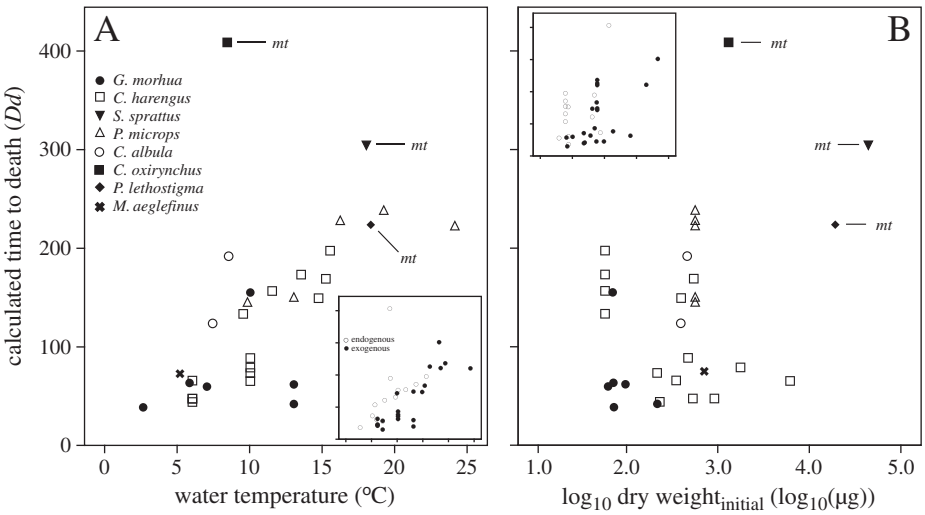

Fig. 7. Time to death (*Dd*) based on the percentile approach (y axis) plotted against temperature (°C; x axis; Panel A) and $\log_{10}$-initial dry weight ($\log_{10}$(µg) ; x axis; Panel B). Symbols indicate species (see figure legend); *mt* indicates muscle tissue *sRD* assays. Inserts: The same plots (same x- and y-axis) with symbols indicating endogenous yolk-sac (white fill) and exogenous (black fill) life stages.

deprivation, when species- and life stage-specific responses are evident that likely reflect different adaptive strategies during starvation.

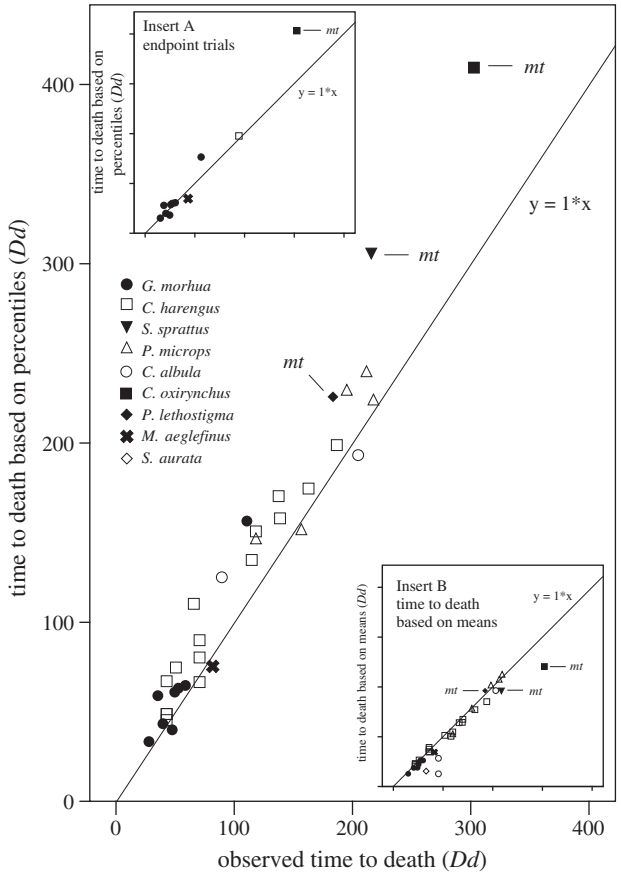

Fig. 8. Time to death (*Dd*) based on the percentile approach (y axis) plotted against observed time to death (*Dd*; x axis). Symbols indicate species (see figure legend); *mt* indicates muscle tissue *sRD* assays. One-on-one line (y = 1 x) for visual support; points to left of this line: time to death is underestimated and mortality not imminent; points to the right: time to death is overestimated and mortality has already occurred. Insert A: The same plot (same x- and y-axis) showing only endpoint trials, i.e. ending with 100% larval mortality. Insert B: Time to death, based on means (*Dd*) (y axis) plotted against observed time to death (*Dd*) (x axis).

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 intended to be a temperature-corrected metric. This implies that at higher temperatures fasting larvae of similar size and life stage exhaust their energy reserves later. It can only be speculated if this is a systematic trend that is caused by failure of the degree-day approach to normalize responses at different temperatures or whether this trend is rooted in life-stage and species-specific thermal sensitivity. Although still not common, the normalization of temperature-effects has helped researchers to reveal causal trends in poikilotherm metabolic rates (Fuiman et al., 1998; Neuheimer and Taggart, 2007). It was also described that for certain behaviorally controlled traits (e.g., swimming activity, prey ingestion and hence the ability to avoid death from starvation) considerable energy is invested to maintain high trait performance across a broader range of temperatures (Dell et al., 2011). A constantly high trait performance over a broad range in temperatures would be impossible to normalize with a simple numerical approach like the *Dd*-normalization. The current dataset is too limited to draw conclusions on the general mechanisms acting here. Further research on the thermal sensitivity of *sRD* in fish early life stages, especially comparing taxa with steno- and eurythermal tolerance ranges, is needed.

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 yolk 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. The window of opportunity is the time period between first feeding (closely related to mouth-gape opening and onset of foraging

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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 to be 5.6 days at 10 °C (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 yolk 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 lipids within hepatic tissues) that could be used to increase starvation resistance. Very short times to death for cod larvae at exactly this transitional life stage were determined in our analysis (on average, well below 50 *Dd*). The rapid starvation rates as well as the highly variable condition levels of transitional life stage cod larvae in all trials were clear indicators of their pronounced vulnerability to starvation.

In sharp contrast to the larval life stage, juvenile sprat (25 to 35 mm length) exhibited high starvation resistance and were an exception to the usual exponential decrease in condition of individuals with time of food deprivation. Sprat deprived for 12 days were not yet beyond their point of no return and all individuals were able to successfully reinitiate feeding and growth (Peck et al., 2004b). The individual starvation rate trajectory of these juvenile sprat indicated that they have a different strategy to respond to food deprivation, compared to the linear decrease in *sRD* condition until mortality that we found for larval fish. Meta-analysis research investigating respiration rates in larval and later stage fish (Bochdansky and Leggett, 2001) demonstrated that metabolic control changes throughout ontogeny in a fashion not solely attributable to body size and storage tissue mass. After prolonged periods of starvation, clupeids (such as sprat and herring) display a continuous loss of body weight and decreasing somatic condition factors (*DW* per unit length) yet only show very modest declines in length (Hauss, 2008).

The 10th percentile *sRD* exhibited a significant negative trend with temperature in yolk-sac and exogenous feeding larvae, with lower 10th percentile *sRD* values at higher temperatures. Exogenously-feeding larvae had a consistently higher 10th percentile *sRD* than yolk-sac larvae at the same temperature. This 10th percentile *sRD* metric represents an important empirical approximation for the lowest species- and life stage-specific *sRD* level under starvation. Besides *sRD* (biochemical condition) larval growth rates are also affected by food depletion. It is assumed that growth rates decrease in a similar fashion as *sRD*, irrespective of species and life stage. This assumption is based on the relation between growth and condition, previously described by a multi-species model relating growth rate and *sRD* with respect to temperature in well-nourished larvae (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) and most research seeks to deal with well-feeding and growing organisms (i.e., when rates of anabolism exceed catabolism). Negative and positive growth rates are always examined in concert, implying a basic assumption that there are no major physiological differences in growing or mildly fasting animals, even though this is usually not explicitly stated. For our data, it was not possible to use the Buckley et al. (2008) growth model to predict (negative) growth rates for food-depleted larvae. We calculated growth rates from the 10th percentile *sRD* values of exogenous life stages from our data set that ranged almost equally from positive to negative, with a mean just slightly above zero. In the absence of external sources of nutrition and without endogenous reserves, the larvae in our dataset were clearly not capable of somatic growth, but this assertion was not supported by the *sRD* growth model.

When the same calculation was done for the 10th percentile *sRD* of yolk-sac larvae (i.e., when most of the endogenous reserves were already exhausted), growth rates were mainly negative, i.e. individuals in this life stage at the 10th percentile *sRD* were correctly identified to be in poor condition. Young yolk-sac larvae (prior to any "mixed feeding period"), totally rely on their endogenous yolk reserves for growth and development. *RD* values are initially high in the yolk-sac stage and have led to overestimates of somatic growth rates using *RD*-temperature models that had been successfully fitted to exogenous-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 substantially underestimated if yolk mass is not excluded from the calculation (Buckley et al., 2006). *RD* can be an indicator of recent growth in yolk-sac larvae when protein accretion in the larval body is considered independently from weight loss due to yolk absorption. For the whole larval body, including the yolk reserves, maternal effects are important in imparting variability to nutritional condition of larvae. 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 can have a profound impact on starvation resistance.

A special case among yolk-sac larval strategies is apparent for North Sea houting (Malzahn et al., 2003). The authors determined that hyperplasia, an increase of cell numbers rather than cell size, characterized the development in this species up to 250 degree-days post hatch. Using the same muscle tissue *sRD* dataset, we demonstrated in our analysis that the endogenous-feeding houting larvae exhibited a) the lowest condition threshold (10th percentile), which was presumably caused by low RNA concentration in the hyperplasia muscle tissue and b) the longest time to death, which was possible because of high levels of maternally-derived yolk reserves. Houting and vendace, the only freshwater taxa in the dataset, are both coregonids in the family Salmonidae, which generally are large at hatch and have large yolk reserves. They can accomplish a greater proportion of early ontogeny by feeding on endogenous yolk reserves. The hyperplasia mode of growth is very efficient for taxa having this life history strategy because it does not require any external food intake for ontogenetic progress. The hyperplasia mode is an adaptation to spawning and growing in a low temperature and/or food limited environment where it is advantageous for larvae, in addition to being large at hatch, to be well developed when either suitable temperatures for growth or adequate food sources are not available until later in the season. Vendace, for example, spawn in the winter and embryonic development can take place over a five-month period at low water temperatures (Karjalainen et al., 1991).

Tissue types may affect *RD* values. Tail and trunk muscle sections were used for *sRD* analysis in some of the experiments in our study. Olivar et al. (2009) systematically investigated the different contributions of tissue type *RD* (e.g., head, eyes, muscle, gut) relative to whole body *RD* and found that muscle tissue had consistently higher *RD* values than other tissues. This result was noted for pre- and post-flexion larval stages of two clupeids (5.7–30.8 mm) collected at sea and laboratory food deprivation trials with a paralichthyid (5.6–7.5 mm) species (Olivar et al., 2009). Muscle tissue is the most important energy-storage tissue in late pre-metamorphosis larvae, prior to stages in which lipid storage becomes important. Muscle growth is highly correlated with *sRD* because of its high protein synthesis rate. At the onset of food deprivation, protein turnover rates in muscle tissue decrease and protein reserves are mobilized to satisfy catabolic needs, based on histological or cell-cycle analysis in fish larvae (Catalán and Olivar, 2002; Catalán et al., 2007). This process proceeds with a reduction of ribosomal RNA and an increase of DNA content per unit dry weight (Bergeron, 1997). Therefore, the starvation signal can be strongly expressed in muscle tissue even though it only accounts for a part of the physiological response in whole body samples. For four species investigated by Olivar et al. (2009), the authors suggested a correction factor to account for tissue-type effects. It remains to be clarified how the decline in relative DNA content per unit dry weight throughout ontogeny, caused by formation of low-DNA organic matter such as bones and lipid storage tissue (Suthers, 1998), is differentially expressed

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in species with different morphometric growth strategies (Froese, 1990).

Larvae analyzed in our research exhibited some body size- and stage-dependent differences in starvation rate and thresholds, but the ability to resist starvation remained generally low over several orders of magnitude in dry weight. Miller et al. (1988) found that taxa with small larvae are more susceptible to starvation than taxa with larger larvae. Folkvord et al. (2009) suggested a trade-off between levels of energy storage and growth rate where species with faster (slower) growing early life stages had little (more) starvation resistance but developed more rapidly (slowly) through life stages that were more vulnerable to predators. Jordaan and Brown (2003) identified clear tradeoffs between body size, growth performance and starvation resistance. In their laboratory study, cod larvae ~12 mm *SL* had the highest potential for growth but also starvation-induced mortality. In contrast, first-feeding cod yolk-sac larvae have been demonstrated to have 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 pass through multiple "critical periods" where starvation resistance and growth capacity are linked. For the present dataset, the percentile approach improved the interpretation of *sRD* values of heterogeneous groups of larvae of different life stages and body sizes under food depletion. The a priori assumptions about the sampled population (stochastic distribution), the function describing the decline of *RD* over time of food depletion (exponential) and the selective mortality of individuals in low condition (edge of death), enabled the percentile approach to better represent the physiological process of starvation.

4.3. Population percentiles and percentile approach

A key premise of this study is that variability among individuals in growth potential and starvation resistance strongly determine responses by fish larvae cohorts to environmental conditions. It is necessary to move beyond simply using group averages in nutritional condition to characterize a cohort, either in the laboratory or in the field because the group-average metric does not accurately capture the heterogeneity in condition typically exhibited by fish early life stages. If we are to develop predictive models to describe how larval condition relates to survival and growth at later life stages, we must better define the distribution of the characteristics in individuals, for example values of condition indices, starvation resistance, and growth potential.

Monitoring individual larvae over time, even under controlled laboratory conditions, is extremely difficult. However, inferential methods can be developed such as those proposed by Folkvord et al. (2009), who used cumulative size distribution to derive subpopulation 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 relative size of a larva compared to other individuals of the same cohort is not likely to change in the short term; for example, ranks of cod larvae remained the same from yolk-sac to metamorphosis stage (Paulsen et al., 2009). Thus comparing larval growth is best achieved by comparing larval size from the same percentile of a population at consecutive samplings rather than comparing an individual's size with the mean size in the previous or following sampling. The same situation may be the case for larval condition, where condition of an individual on a given day will depend to some extent on its condition on the previous day. In the case of *RD*, the change in the RNA content of a larva is likely to be more important with respect to the short term change in biochemical condition because total DNA content, reflecting the number of cells in an organism, will not change dramatically from one day to the next (Clemmesen, 1994).

Repeated measurements of condition to determine an individual's condition trajectory under food depletion is virtually impossible 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 a condition index or proxy (e.g., responsiveness, sensitivity), the direction of change in an individual's condition may not be apparent. This is especially true for *RD* that provides the *ad hoc* status of protein synthesis rate and recent growth rate, but does not indicate the direction of change in condition over time.

The percentile approach is a first step to overcome the inability to identify individual starvation trajectories and it provides a metric not otherwise available from population means. It must be realized though, that in our research the same dataset was used for development and application of this approach. Because these steps are not independent, careful validation was mandatory. Independent datasets were lacking and cross-validation, for example by step-wise exclusion of individual experiments and validation against the remaining experiments, would have required substantially more data. We therefore compared the percentile-based starvation parameters with the traditional and conservative approach of population mean-based estimates of *RD*. For starvation rates, we found that these two approaches produced almost identical results. This indicates that the percentile- and mean-based regression slopes of condition (*sRD*) against time of food deprivation were parallel in most trials and were only slightly off-set (higher intercept in 75th percentile approaches). Most of the experiments applied sampling schedules designed for pooled estimates of *sRD* from replicate tanks, and presumably sampled only as many individuals from each tank as needed to obtain a "good" arithmetic mean. The predictive power of the percentile approach can be expected to increase with increases in the number of samples drawn from the experimental population. Stabilization of the 75th percentile, i.e. when it changes not too abruptly over time of food depletion, suggests itself as a good indicator for sampling power. Both the 90th and the 10th percentiles of the sampled population proved to be valid approximations of larval condition at onset of food deprivation and the edge of death, respectively. Applying this finding, based on experimental populations in the laboratory, to populations in the sea it is possible to circumvent labor-intensive approaches to identify duration of starvation time in individuals, for example by measuring otolith increment widths (Baumann et al., 2005) or from histology (Ehrlich et al., 1976; Gisbert and Doroshov, 2003).

The calculated time to death was successfully validated with a rigorous selection of trials that terminated with larval mortality. Time to death, based on the percentile approach, slightly underestimated observed time to death (or more correctly: trial duration), which was expected because most individuals in the 10th percentile fractions were not ultimately close to death. Experimental trials were terminated because of pre-determined experimental schedules and presumably the actual edge of death would have been reached later and with a lower 10th percentile value of *sRD*. In contrast, the population mean-based calculation overestimated trial duration in most cases. We believe that, for these laboratory experiments, the percentile approach was successfully validated and that it provides an accurate approximation of *sRD* starvation parameters at the level of individuals.

The percentile approach is relatively straightforward in laboratory research but more difficult to apply in the sea. In field research, it is necessary to derive the *sRD* metrics (10th, 75th, and 90th percentiles), and to relate them not only to life stages, but also to population and habitat characteristics. These 10th and 90th percentile metrics have been previously applied in evaluations of marine fish early life stages. Clemmesen et al. (2003) reported that the distribution of *RD* values and patterns of percentiles of *RD* were strongly influenced by environments experienced by larvae in mesocosm research. Individuals in a mesocosm with warmer temperatures and higher prey concentrations had higher larval growth rates and *RD* values, and relatively large prey in their gut

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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 10th percentile value of *RD* was stable with time in the warm-high prey mesocosm, indicating a non-selective environment (predators were absent) whereas it increased rapidly during the first three weeks in the cold-low prey mesocosm, suggesting that only the fittest larvae were able to survive and successfully compete for food in the cool, prey-limited environment.

Evaluating changes in specific percentiles of *RD* has also yielded insight into how poor feeding and starvation act to control early life stage survival in the sea. In research on sprat larvae in the Bornholm Basin (Baltic Sea), Voss et al. (2006) reported that stable but relatively high 10th percentile values of *RD* with increasing body size reflected a feeding environment with high copepod nauplii concentrations that was favorable for small, first-feeding sprat larvae in April and May. Lower 10th percentile values of *RD* occurred in July, suggesting a less selective environment in which both fast-and slow-growing larvae survived. In the April–May period, larvae in lower nutritional condition suffered high mortality and were removed from the population, resulting in a rise in the 10th percentile. A similar increase in the 10th percentile values of *RD* was observed by Huwer et al. (in press) who examined nutritional condition of Baltic cod larvae during a period of prey limitation. These results support the proposal that during periods of abundant food supply, selective pressure for fast growth is relaxed and slow-growing larvae may experience improved survival (Meekan and Fortier, 1996).

4.3.1. Perspective

An empirical parameterization of the functional model describing changes in nutritional condition under food depletion will advance understanding starvation prevalence in the sea and will answer the questions: When and where are early life stages of fish pre-recruits exposed to starvation in the sea? How important is starvation as a cause of mortality? There is a substantial body of literature that indicates limiting prey levels are a source of larval mortality and a factor affecting recruitment success. In the Baltic Sea, empirical and model results indicate prey limitation controls survival of cod larvae (Köster et al., 2003). In a recent study, Huwer et al. (in press) utilized *sRD* measurements to identify Baltic cod larvae in poor nutritional condition within areas having low concentration of the preferred prey, *Pseudocalanus acuspes*. In the Northwest Atlantic, Buckley et al. (2010) identified 'windows for survival' of Atlantic cod and haddock larvae based upon seasonal and inter-annual differences in larval *RD* condition and prey abundance. Voss et al. (2006) utilized *sRD* measurements to reveal size-specific 'windows of survival' for Baltic sprat larvae that were linked to the availability of suitable prey. These results support Cushing's 'match–mismatch' hypothesis (Cushing, 1974; Cushing, 1990) which emphasizes the key role of timing of prey production and its coincidence with early life stages of fish as a mechanism controlling recruitment in marine fishes. Our efforts to quantify starvation rates and times to death based upon thresholds in biochemical indices (*RD*) will contribute to progress in evaluating and understanding how prey limitation acts to affect early life stages. The advantage of our proposed *sRD*-derived measure of mortality risk is that it may be applicable as a first estimate of prey limitation effects in many environments and across a wide range of species.

5. Conclusion

Our analysis has shown that starvation-induced changes in condition in pre-recruit life stages of fishes can be described by a common function when temperature effects, 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 biochemical condition (standardized RNA–DNA ratio) in sampled populations to derive an estimate for starvation rates and mortality thresholds of biochemical condition on the level of 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 in condition, mortality threshold, and time to death) based on data from controlled laboratory trials. Although the selective loss of individuals in poor condition will undoubtedly differ between the laboratory and sea, 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 of how different life stages and/or species are able to cope with periods of prey deprivation and how frequently cohorts of fish experience life "on the edge of death".

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