Pre-settlement processes of northern rock sole (*Lepidopsetta polyxystra*) in relation to interannual variability in the Gulf of Alaska

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ABSTRACT

Understanding the effects of climate variability on growth dynamics and timing of early life history events in marine fishes can provide insights into survival, recruitment and productivity. We examined interannual variation in indicators of larval growth rates, size at hatch and metamorphosis, and the timing of metamorphosis of northern rock sole (*Lepidopsetta polyxystra*) over 5 years in two nurseries at Kodiak Island, Alaska, USA. Variation in early life characteristics was quantified using laboratory-validated otolith structural analysis and related to water temperature and spring bloom dynamics in the Gulf of Alaska. Overall, results indicated

that temperature contributed more to interannual variation in northern rock sole growth, size and phenology patterns than phytoplankton dynamics. Size at hatch was positively related to winter-spring spawning temperatures. Larval growth metrics were generally consistent with thermal effects as temperatures above 4°C appear necessary, but are not sufficient to support rapid growth. Reflecting the cumulative effects of temperature, the timing of metamorphosis was related to both seasonal and interannual variation in temperature with earlier dates of metamorphosis in warmer years. Conversely, fish size at metamorphosis was similar across years, suggesting that the competency to metamorphose is related to attainment of a minimum size. These results demonstrate the important role of temperature in regulating early life history phenology of northern rock sole and suggest that temperature-driven phenological shifts may also influence the time of spawning and hatching.

Highlights:

- We quantified variation in early life history characteristics of northern rock sole
- Timing of metamorphosis was related to temperature variability
- We observed a constraint in the variation in fish size at metamorphosis
- Early life history characteristics were not related to phytoplankton production

1. Introduction

High rates of mortality in the egg and larval stages of marine fish indicate that small changes in early life history characteristics have the potential to influence survival to settlement in nursery grounds (Bailey and Houde, 1989; van der Veer et al., 2000). For species with complex life histories such as flatfish, the physiological process of metamorphosis and associated behavior of settlement are critical transitions that link the pelagic larval stage to the benthic juvenile stage. The timing of metamorphosis and settlement as well as fish size at these transitions reflect variation in growth and development in earlier life stages (Pepin and Myers, 1991). Therefore, processes driving metamorphosis and settlement are important for understanding connectivity between life history stages because larval traits may be correlated with traits exhibited during post-settlement life stages (Chambers et al., 1988).

Variation in early life history traits is often related to environmental variables such as temperature. In ectotherms, water temperature is a direct determinant of vital rates, including growth and development (e.g. Pepin, 1991; Meekan et al., 2003; Sponaugle et al., 2006), as well as an indirect determinant of the timing of early life history events, such as spawning, hatching, and settlement (e.g. Genner et al., 2010; Fincham et al., 2013). Variability in temperatures during the larval stages can affect growth rates, which determine larval stage duration and the subsequent timing of metamorphosis and settlement (Benoît et al., 2000). However, the extent to which early life history processes vary under different environmental regimes remains poorly understood. Thus, knowledge of growth dynamics in relation to shifting climatic conditions is critical to understanding recruitment fluctuations and survival of marine fish.

In addition to temperature, larval food supply plays an important role in the early life history as low prey levels can limit larval growth and development (Houde, 1989).

Phytoplankton dynamics can influence productivity of higher trophic levels, linking environmental conditions and early life history characteristics. Documented interannual variability of phytoplankton blooms in relation to thermal shifts could lead to asynchrony with the prey resources necessary for larval growth and development to metamorphosis (Mackas et al., 1998; Edwards and Richardson, 2004). Metamorphosis is an energetically demanding process and it is likely that larval fish must meet some energy storage requirement prior to initiating metamorphosis (Boglino et al., 2012; Fraboulet et al., 2010). Spatial and temporal mismatches with prey may have negative implications for larval growth as well as energy stores prior to metamorphosis (Laurel et al., 2011). Alternatively, warmer waters promoting faster growth may allow larvae to take advantage of earlier bloom conditions. Overall, a better understanding of the links between larval growth, phenology and seasonal production of lower trophic levels is necessary.

Northern Pacific marine ecosystems are some of the most productive in the world, supporting high abundances of many commercially important groundfish species (Mundy, 2005). Large-scale regime shifts and recent warming trends in the Gulf of Alaska may have direct and indirect effects on early life history characteristics of fish species in the North Pacific (Royer and Grosch, 2006; Laurel et al., 2011). While much effort has focused on understanding early life dynamics of walleye pollock (*Gadus chalcogrammus*), less work has addressed other commercially important species such as northern rock sole (*Lepidopsetta polyxystra*). Supporting the second largest flatfish fishery in the United States, northern rock sole (NRS) is abundant in the Gulf of Alaska and Bering Sea (NMFS, 2013). Adult NRS spawn demersal eggs from mid-winter to spring in bays and coastal areas at an average bottom depth of 45 m (Stark and Somerton, 2002). After a pelagic larval stage, juveniles metamorphose and settle in shallow,

sandy coastal habitats in June and July (Norcross et al., 1995; Laurel et al., 2015).

Post-settlement processes in NRS are related to temperature during the spawning and larval period (Hurst et al., 2010); however, pre-settlement processes and the relative importance of environmental variables on early life history characteristics have not been quantified. Previous research suggests that NRS are a 'cold-adapted' species due to their ability to maintain positive growth at temperatures below 2°C (Hurst and Abookire, 2006). However, like most other cold-water species, growth rates of NRS larvae increase with temperature to some threshold, above which growth rates decline (Ryer et al., 2012). Furthermore, faster larval growth in elevated temperatures results in a shorter larval stage duration and earlier settlement at larger sizes in laboratory settings (Laurel et al., 2014). Despite the expected links between temperature and the processes of metamorphosis and settlement, relatively few studies have explored the direct and indirect effects of environmental conditions on phenology in wild flatfish populations. Doyle et al. (2009) reported a negative correlation between NRS larval abundance and Gulf of Alaska sea surface temperatures during spawning periods in a 21-year ichthyoplankton time series. However, these temperature-related shifts in larval abundance are hypothesized to be due to phenological shifts in larval production, and specifically, shifts in the time of spawning and hatching in relation to the timing of the annual research survey. Additionally, observed interannual differences in larval size from field collections suggest later spawning in response to cooler water temperatures (Lanksbury et al., 2007). Potential temperature-driven shifts in the phenology of metamorphosis and settlement of NRS also have yet to be determined.

The results of these prior studies suggest that field-collected NRS larvae may exhibit faster larval growth and development as well as an earlier onset of metamorphosis and settlement at larger sizes in response to warmer thermal regimes in the Gulf of Alaska. However, if food

availability directly influences growth in the field, then fast growth and large size of larvae may be associated with the timing and magnitude of the spring bloom. Alternatively, both temperature and food availability could influence larval characteristics with the relative importance of these factors varying across years. In this study, we validated otolith structural landmarks and daily increment deposition using laboratory-reared NRS. We then combined field collections of NRS juveniles in two nursery sites in the Gulf of Alaska with otolith structural analysis to determine the variation in early life history characteristics across years (2005, 2007, 2009-2011) and related early life history processes to environmental conditions. We quantified interannual variation in size at hatch, larval growth, size at metamorphosis, and the timing of metamorphosis of NRS. We also determined if size and timing of metamorphosis were related to growth during the larval period. In an effort to disentangle sources of early life history variation, larval NRS characteristics were compared with near-surface temperature and phytoplankton productivity estimates in the Gulf of Alaska.

2. Materials and methods

2.1 NRS otolith structure and daily increment validation

While otolith growth patterns often coincide with developmental events, the timing of formation of these otolith landmarks in relation to morphological development differs among flatfish species (Toole et al., 1993; Modin et al., 1996; Neuman et al., 2001). Therefore, otoliths of laboratory-reared NRS were used to identify structural landmarks and determine relationships to specific life-history events. Fish used for validation were provided by the Fisheries Behavioral Ecology Program of the Alaska Fisheries Science Center (AFSC) from ongoing laboratory cultures (see Laurel et al., 2014 for details on culture conditions).

NRS larvae were collected 0 to 2, 4, and 6 d post-hatch, euthanized with tricaine

methanesulfonate (MS-222), and preserved in ethanol for verification of a hatch check.

Polished sagittae ($n = 25$) displayed a check at ~15-18 μm in diameter, which corresponded with

hatching, based on the number of post-check increments. To determine if a check corresponds

with metamorphosis and/or settlement, preserved fish from experiments were selected based on

the stage of eye migration. Five to ten individuals from each stage were examined based on

Ryland's (1966) morphological classification of larval plaice, starting with initial eye migration

and concluding with full pigmentation and complete eye migration. Otoliths were mounted,

polished, and imaged. Otoliths from bilaterally symmetric larvae were spherical in shape (Fig.

1a). The initiation of eye migration corresponded with the formation of an accessory growth

center along the otolith circumference (Fig. 1b and 1c), with additional accessory growth centers

forming when the migrating eye reached the dorsal edge of the head (Fig. 1d). Completion of eye

migration corresponded with full pigmentation, dorsal fin morphology (i.e. the dorsal fin reaches

the migrating eye) and deposition of a check at the edge of "petal-like" protrusions formed by the

accessory growth centers (Fig. 1e and 1f). This "metamorphic check" was observed in all

laboratory reared age-0 NRS individuals with completed eye migration (SL range = 10.90 -12.43

mm, mean metamorphic check width = 284.88 μm ± 24.61 μm, $n = 30$). This check was also

consistently observed in otoliths of field-caught juvenile NRS although at a larger otolith size

(mean check width = 328.50 μm ± 32.28 μm, $n = 357$). Daily increments could not be

enumerated through the region of accessory primordia deposition, meaning that age (post-hatch)

of post-metamorphic fish could not be determined. Otolith size was positively correlated with

body size in both recently hatched larvae ($r = 0.79$, $p < 0.001$, $n = 30$) and in fish prior to and

during metamorphosis ($r = 0.87$, $p < 0.001$, $n = 35$) demonstrating the validity of applying otolith

size as a proxy for fish size at these life history transitions.

In the laboratory, NRS that remained on the bottom of the tank were characterized as "settled" (Laurel et al., 2015); however, they were often still in the early to mid-stages of eye migration. This observation suggests that settlement behavior may not be synchronous with completion of eye migration and formation of the otolith check, at least in laboratory culture. Therefore, the observed otolith check was considered a metamorphic check.

Age-0, post-settlement, laboratory-reared NRS otoliths were used to validate the daily deposition of increments. Increment deposition was examined in fish raised within controlled rearing temperatures and feeding conditions prior to a 7°C drop in water temperature. Image analysis revealed a clear check near the otolith edge. Two independent counts of consecutive daily increments from this check to the otolith edge did not differ from the number of days since the temperature decline (mean = 15.0 ± 1.0, t-test, $p > 0.05$, $n = 14$), confirming daily increment formation in this species.

2.2 Fish collection

Fish were collected at two sites off the northeast coast of Kodiak Island, Alaska, USA (Fig. 2). Holiday Beach (57° 41.2' N, 152°27.7' W) in Middle Bay and Pillar Creek Cove (57° 49' N, 152° 25' W) in Monashka Bay, are known nursery grounds for age-0 NRS (Stoner et al., 2007). Age-0 NRS have been sampled annually in July and August at both sites by the Fisheries Behavioral Ecology Program of the AFSC since 2004 (Hurst et al., 2010). Previous sampling indicated that peak settlement likely occurs in May-June (Hurst and Abookire, 2006; Laurel et al., 2015). Sampling was conducted with a 2-m beam trawl with a 3-mm mesh codend at fixed transects. Three to five 5-minute trawls were conducted parallel to the shoreline at approximately

10-m depth intervals between 7 and 30 m depth on each sampling day (Hurst et al., 2010). After each tow, surface and bottom temperature, salinity and oxygen concentrations were measured at the mid-point of the tow (YSI model 85). Trawl catches were identified to species, frozen, and shipped to the AFSC laboratory in Newport, OR, U.S.A. Archived NRS collections from field sampling in 2005, 2007, 2009, 2010 and 2011 were selected for analysis due to adequate sample sizes and suitable otolith condition (Table 1). Otolith degradation during storage prevented the inclusion of additional years and resulted in relatively small sample sizes for 2005 ($n = 60$) and 2007 ($n = 69$).

2.3 Environmental parameters

To characterize interannual variation in temperature during the pelagic larval period, data were extracted from hydrographic station GAK-1 (http://www.ims.uaf.edu/gak1/) from 1 January to 1 May. Located near Seward, Alaska (~59°51'N, 149°28'W), ~180 miles northeast of Kodiak Island nursery sites, the GAK-1 time series includes water temperature and salinity readings every 15 minutes. GAK-1 daily temperatures are well correlated with other regional temperature records, indicating that GAK-1 records reflect general patterns of temporal variation in temperatures in the Gulf of Alaska. As the highest abundances of NRS larvae are found at depths of 10-30 m (Lanksbury et al., 2007), water temperatures measured at 20 m depth were used in analyses. The 15-minute temperature readings were consolidated into 15-d means.

For analyses during the metamorphic period (mid-March to mid-July), we used the hourly temperature record at 10 m depth in Trident Basin on the NE coast of Kodiak Island (Fig. 2). Short-term temperature records for Holiday Beach and Pillar Creek indicated that the Trident Basin temperature record was consistently within the range of temperatures measured at the

sampling sites (Hurst et al., 2010). Therefore, Trident Basin temperature records were used to describe mean temperatures in the nursery areas in 15-d intervals.

To describe interannual variation in timing and magnitude of the spring bloom, satellite-derived chlorophyll-*a* (chl-*a*) concentrations were used as a proxy for phytoplankton biomass and bottom-up production in the Gulf of Alaska. Chl-*a* data were extracted at 9-km spatial resolution from Level-3 MODIS-Aqua 8-day composite time series data for the grid surrounding the Kodiak Island study area (56-60°N, 148-155°W) and used to determine the onset date (Bloom$_{Onset}$) and magnitude (ChlA$_{Max}$) of the annual spring bloom (see Fedewa 2015 for details).

2.4 Preparation and analysis of field-collected juvenile NRS otoliths

Field-collected juvenile NRS were thawed and measured (SL, nearest 0.1 mm). Sagittal otoliths were removed and photographed. Image analysis software (Image-Pro Premier®) was used to measure otolith length (anterior to posterior: longest axis) and width (dorsal to ventral: longest perpendicular axis). Right sagittal otoliths were used for all analyses. A subset of 30-50 otoliths from each month (July and August) and year (2005, 2007, 2009-2011) at each of the two nursery sites (Holiday Beach and Pillar Creek Cove) was selected for analysis. Otoliths were mounted, polished and imaged with a Leica DC300 camera and Leica DM1000 compound microscope (40-400x magnification).

Daily increment widths were measured to reflect growth rates whereas the diameter at the hatch check (HC$_W$) and metamorphic check (MC$_W$) were used as proxies for fish size at these life history events. The mean of 10 increment width measurements adjacent to designated otolith landmarks were used to characterize early larval (EL$_{IW}$), larval (L$_{IW}$), and post-metamorphic (PM$_{IW}$) growth rates in subsequent cross-sectional data analyses (Fig. 3). Ten consecutive otolith

increments were measured for each growth metric and used for longitudinal data analyses.

Separate measures were made for early larval growth and larval growth rates due to the consistent presence of a check ~45 µm in diameter and an associated increase in increment width (0.8 µm to 1.2 µm average increment width), likely coinciding with notochord flexion (Jenkins, 1987; May and Jenkins, 1992). Enumeration of increments from hatch check to the 45-µm check in individual otoliths with clear microstructure ($n = 30$) indicated that this check is formed approximately 20-30 d post-hatch. However, given that increments near the hatch check were often indistinct, the last 10 increments prior to the 45-µm check (i.e. ~10-20 d post-hatch) were measured and used as a metric of early larval growth (Fig. 3b). Larval growth was characterized by the first 10 increments following the check. Daily increments representing post-metamorphic growth immediately following eye migration were measured from the ventral edge of the metamorphic check towards the otolith edge (Fig. 3a). This post-metamorphic growth metric (PM_{IW}) was measured across a 10-increment range during the first 20 to 30 d following eye migration.

The date of metamorphosis was estimated for each fish. However, due to difficulties in resolving daily increments continuously from the metamorphic check to the edge of the otolith, empirical estimates for days post-metamorphosis could be directly determined for ~15% of analyzed otoliths ($n = 49$). Therefore a model was developed to estimate days post-metamorphosis (D_{PM}) from the subset of otoliths with direct counts of days post-metamorphosis based on mean post-metamorphic increment width, (PM_{IW}) and cumulative post-metamorphic ventral otolith growth, (V_{OG}; $R^2 = 0.90$; Fedewa, 2015; Eq. 1).

$$D_{PM} = 34.986 - 10.942 \cdot PM_{IW} + 0.333 \cdot V_{OG} \qquad \text{(Eq. 1)}$$

Date of metamorphosis (DOM) was then determined for each fish by subtracting estimated days

post-metamorphosis from the known date of capture.

2.5 Data Analyses

Data were tested for normality and homogeneity of variance and log-transformed when necessary. All statistical analyses were conducted in R, version 3.1.2 (R Development Core Team, 2012). Due to missing data in some sites and months, within-year effects of nursery site (Pillar Creek Cove and Holiday Beach) and sampling month (July or August) on larval characteristics were evaluated with Student's t-tests. Bonferroni corrections were used to account for multiple comparisons. Within years, mean size at hatch, mean early larval growth, mean larval growth and mean size at metamorphosis did not vary significantly between nursery sites or sampling month (Student's t-test, $p > 0.05$). Therefore, samples were pooled for each of the growth or size metrics and tested for interannual differences with a one-way Analysis of Variance (ANOVA) and Tukey-Kramer *post-hoc* analyses. Early larval and larval growth trajectories were compared across years with repeated-measures ANOVA tests because consecutive increment widths within individuals are not independent. A repeated measures ANOVA was used to examine variation in growth among increment counts (increment 1 to increment 10) as well as among years. Within-year date of metamorphosis comparisons did vary significantly between nursery sites or sampling months (Student's t-test, $p < 0.05$) so a three-way ANOVA with year, month and site main effects was used.

Relationships between mean size or growth and temperature across years were examined with correlation analyses (Pearson's correlation coefficient). Fifteen-day mean GAK-1 temperatures (herby referred to as larval temperatures) across the time period corresponding with the pelagic larval stage (1 January to mid-March) were compared with size at hatch, early larval growth and larval growth rates. Likewise, 15-d mean Trident Bay temperatures (hereby referred to as metamorphosis temperatures) across the range of metamorphic dates (mid-March to mid-

July) were compared to mean size at metamorphosis and mean date of metamorphosis. To refine these comparisons, pre-metamorphosis temperature (Pre_{Met}) was determined as the mean temperature across the 14-d period prior to the earliest estimated date of metamorphosis in each year. Correlation analysis was also used to determine if size and timing of metamorphosis were related to early larval or larval growth metrics. All α values for correlations were Bonferroni corrected to account for multiple comparisons.

The greatest variation was observed in the timing of metamorphosis; therefore, we examined additional environmental variables in relation to estimated dates of metamorphosis. To describe annual variability in temperatures during metamorphosis, mean temperature across the range of estimated metamorphosis dates was calculated for each year ($T_{MetRange}$). To characterize individual thermal history, the mean temperature across the 14 d prior to each fish's estimated date of metamorphosis was determined (T_{Indv}). In addition, to describe annual variability in productivity regimes, mean chl-a concentration was calculated across the 14 d prior to the mean estimated date of metamorphosis for each year ($ChlA_{PreMet}$). To characterize individual-based chl-a conditions, the mean chl-a concentration across the 14 d prior to individual date of metamorphosis estimates was determined ($ChlA_{Indv}$). The annual date of the spring bloom onset ($Bloom_{Onset}$) was also included in analyses.

To quantify the relative influence of these environmental variables on the timing of metamorphosis, multivariate linear regression models were fit and evaluated with Akaike information criteria adjusted for small sample size (AIC_c; Burnham and Anderson, 2004). Due to inherent collinearity between temperatures and chl-a concentrations, variable selection for model inclusion was contingent on variance inflation factor values for each predictor ($VIF < 2$) (Zuur et al., 2009). We determined: 1) the difference between the AICc of the best-fitting model and that

of model i (Δ_i); 2) Akaike weight, (w_i); and 3) coefficient of determination, (R^2) (R package 'AICcmodavg'). The relative importance of each model was also assessed to quantify each individual parameter's contribution to the regression model (R package 'relaimpo').

3 Results

3.1 Environmental parameters

Annual minimum larval temperatures (3.1-4.6°C) were consistently observed in February and March with temperatures increasing in mid-April to May. Average larval temperatures in 2005 and 2010 were at least 1°C warmer than average temperatures in 2007 and 2009 (Fig. 4a, one-way ANOVA, $p < 0.001$), with 2007 being one of the coldest winters in the central Gulf of Alaska in the past 40 years. Nursery ground temperatures steadily increased from April to July. Whereas 2010 was the warmest year in the study period during the larval period, 2005 was the warmest year during the metamorphosis period (on the nursery grounds). Nursery temperatures in 2005 were well above the long-term average at this site, averaging at least 1°C warmer than those in any other study year, and at least 1.5°C warmer than 2007 and 2009 temperatures (Fig. 4b, one-way ANOVA, $p < 0.001$).

Annual maximum chl-*a* concentrations were reached by mid-May (max range 2.50-7.07 mg m^{-3}) (Fig. 4c). Following the peak of the spring bloom, chl-*a* concentrations decreased until June when another moderate bloom occurred (max range 2.18-5.59 mg m^{-3}). Chl-*a* concentrations declined in July from the annual peak but remained elevated for the duration of the summer period. The largest spring bloom occurred in 2009 (max concentration 7.07 mg m^{-3}) and the smallest occurred in 2007 (2.50 mg m^{-3}). The earliest date of bloom onset occurred in 2011 (3 April) while the latest date of onset occurred in 2009 (7 May).

3.2 Size at hatch, early larval and larval growth

Size at hatch differed among years with a significantly larger mean size at hatch in 2010 than in 2007 and 2009 (Fig. 5a, Kruskal-Wallis ANOVA, $p < 0.001$). Mean size at hatch was positively correlated with several of the 15-d temperature means periods (Table 2, r-values 0.69 – 0.93), but none of these were significant after Bonferroni correction for multiple tests.

Log-transformed mean early larval growth also differed significantly across years, with larvae exhibiting faster growth in 2005 than all other years (Fig. 5b, ANOVA, $p < 0.001$). Longitudinal analysis of early larval growth trajectories revealed a significant effect of increment number, as increment widths consistently increased from increment 1 to increment 10 (with increment 10 being immediately adjacent to the 45 µm check; repeated measures ANOVA, $p < 0.001$). There was also a significant year effect, with larvae growing faster in 2005 than any other year ($p < 0.01$). Interannual comparisons of mean larval growth indicated that growth was faster in 2005 than in 2007 and 2011 (Fig. 5c, Kruskal-Wallis ANOVA, $p < 0.001$). Results were similar for longitudinal data analysis of larval growth. There was a significant interaction between increment number and year on increment width ($p < 0.05$) as increment widths in 2005 increased more slowly away from the 45-µm check than the other years. In addition, there was a significant effect of increment number as increment widths increased away from the check ($p < 0.001$) and a significant year effect ($p < 0.05$). Post-hoc tests indicated that larval growth was significantly faster in 2005 than in 2011 ($p < 0.01$).

Mean size at hatch was not correlated with chl-a concentrations. Contrary to the hypotheses, annual mean early larval growth and larval growth were not significantly correlated with any of the 15-d mean temperatures or 8-d mean chl-a concentrations (Table 2, all $r \leq 0.60$). However, the lack of strong correlations between water temperatures and growth rates during the

larval period was driven by the observation of low growth rates in 2011 despite relatively warm

conditions (Fig. 6). The remaining years of the study exhibited a clearer relationship between

temperature and growth rates.

3.3 Size and timing of metamorphosis

Overall mean metamorphic check width was 328.50 ± 32.28 µm ($n = 357$) and there was

no significant year effect on size at metamorphosis (one-way ANOVA, $p > 0.05$). Mean size at

metamorphosis was not significantly correlated with spring bloom dynamics or with any of the

15-d average temperatures in each year (Table 3, max $r = 0.86$; 2 July-16 July). Size at

metamorphosis was positively, but not significantly, correlated with mean early larval growth

rate ($r = 0.73$) and mean larval growth rate ($r = 0.74$).

Date of metamorphosis estimates for NRS individuals spanned nearly 4 months from

late-March to mid-July (Fig. 7), with an overall mean date of 21 May. Interannual variation in

metamorphosis timing was characterized by an earlier mean date of metamorphosis in 2005 (4

May) compared to all other years. Mean dates of metamorphosis were later in 2009 (28 May)

and 2007 (5 June) than the other three years (3-way ANOVA, $p < 0.01$). There were significant

interactions between collection month and year as well as month and site on date of

metamorphosis estimates. Site differences were driven by earlier dates of metamorphosis at

Holiday Beach in 2005 and 2007 (site x year interaction, $p < 0.01$). July fish in 2010 had later

dates of metamorphosis than August fish, driving a significant month x year interaction ($p <$

0.01). These site and month effects are further explored in Fedewa (2015).

Metamorphosis occurred earlier in warm years than cold years: the mean date of

metamorphosis was negatively correlated with temperatures 2 weeks prior to the annual onset of

metamorphosis (Table 3, $r = -0.99$, $p < 0.01$) as well as mean temperatures in late April and early May (Fig. 6d, $r = -0.98$, $p < 0.01$). Mean date of metamorphosis was positively correlated with the onset of the spring bloom ($r = 0.65$) although the relationship was not statistically significant. There were non-significant, negative relationships between date of metamorphosis and mean early larval growth rate ($r = -0.66$) and mean larval growth rate ($r = -0.35$). Again, the relatively poor fit of these relationships was driven by the observation of relatively slow early larval and larval growth rates in 2011.

3.4 Variation in timing of metamorphosis in relation to environmental conditions

All five environmental variables were included in candidate models describing the timing of metamorphosis, although multicollinearity (VIF > 2) resulted in exclusion of the majority of interaction terms. Fifteen models were evaluated and results are presented for models with Akaike weight (w_i) > 0 (Table 4). Based on the performance criteria, models containing both temperature parameters T_{Indv} and $T_{MetRange,}$ received considerably more support (M1-M4, $\Delta_i <$ 2.000) than the poorest performing model which included neither temperature parameter (M5, Δ_i = 712.361). Models including spring bloom dynamics (M3-M5) received less support than models without spring bloom dynamics (M1-M2). The best model selected by performance criteria (M1, $w_i = 0.4223$) indicates that the variation in timing of metamorphosis reflects temperature conditions experienced by individuals 2 weeks prior to metamorphosis (T_{Indv}) as well as temperatures during the overall temporal window of metamorphosis across years ($T_{MetRange}$) (Fig. 8). Calculations of the relative importance of variables in each model indicated that T_{Indv} accounted for >70% of the variation in timing of metamorphosis in M1 and > 60 % of the variation in M2-M4.

4 Discussion

We combined multiple years of field collections with otolith structural analysis to document interannual variation in early life history characteristics of NRS and present the first estimates of size and timing of metamorphosis in field-caught NRS. Variation in the timing of metamorphosis for NRS in the Gulf of Alaska was well-described by both seasonal and interannual temperature variability. Conversely, fish size at metamorphosis displayed little variability and appeared to be driven by ontogeny rather than environmental conditions or larval growth rates. These results highlight that temperature variability in the Gulf of Alaska exerts a strong influence on early life history characteristics of NRS.

Temperature is assumed to be one of the primary influences on fish growth with higher temperatures leading to faster growth in larval flatfish (e.g. Benoit and Pepin, 1999; Hutchinson and Hawkins, 2004; Laurel et al., 2014). In this study, the patterns of variation in NRS larval growth were generally consistent with a positive thermal effect. As expected, the fastest larval growth occurred in one of the warmest years in the study period (2005), while slow growth was observed in the cold year (2007). However, several factors reduced the power of these analyses leading to a general lack of significant correlations between growth metrics and temperature measures. Additionally, the occurrence of anomalously slow growth in 2011 resulted in non-significant correlations with temperature across years. This observation of slow growth in a year with moderate temperatures suggests that while warm temperatures may be necessary, they may not be sufficient to promote rapid growth of larval NRS.

There were some limitations in our study related to our inability to determine total age of NRS individuals due to the obscure region corresponding with metamorphosis. Therefore, larval growth increments could not be assigned to specific dates and instead, analyses were based on

temperatures in fixed temporal windows across years. Some researchers (Sogard, 1991; May and Jenkins, 1992; Joh et al., 2011) have relied solely on flatfish lapilli due to the presence of clearer daily increments than observed in sagittae. In NRS, daily increments were consistently clearer in lapilli although there was no evidence of a metamorphosis check or zone, which was a primary focus of our study. In the future, it would be worthwhile to consider examining both otolith structures in order to more fully resolve certain life history questions.

Mean size at hatch was positively related to temperatures during the winter-spring spawning period (although not significantly so after Bonferroni correction). Interestingly, Laurel and Blood (2011) reported that maximum size at hatch of laboratory-incubated NRS eggs occurred at 5°C. We observed the largest hatch size in 2010 when temperatures were 5-6°C on average. Although size at hatch may be influenced by selective mortality in the field that is not observed in the laboratory, these results indicate that there may be a thermal optimum for hatch size in the field as well. In addition to incubation temperatures, maternal effects could play a role in size at hatch in field-collected NRS in the Gulf of Alaska. For example, female condition prior to spawning as well as conditions during spawning and egg stages may influence egg size, fish size at hatch and larval growth (Chambers and Leggett, 1996; Benoit and Pepin, 1999).

There was little interannual variation in size at metamorphosis, indicating that metamorphosis in NRS is a strongly size-dependent process. NRS size at metamorphosis was not associated with larval growth, suggesting that the initiation of life history transitions such as metamorphosis could be more dependent on size than growth rates (Fuiman, 1997). The acquisition of a competent size for metamorphosis as well as a constraint in variation in size at metamorphosis has been noted in other flatfish species (Chambers and Leggett, 1987; Amara et al., 2000; Geffen et al., 2007). Results indicate that metamorphosis is a time of convergence in

the life history of NRS despite interannual variation in hatch size and larval growth rates. Metamorphosis may act as a compensatory processes, dampening variability in size and growth induced from hatch to late larval stages.

If larval growth metrics vary interannually but size at metamorphosis does not, the most parsimonious explanation for the ability of NRS to reach the competent size range for metamorphosis is that the timing of metamorphosis, instead, must vary. Despite fairly protracted metamorphosis dates within each year, there were significant interannual differences in the timing of metamorphosis. Negative correlations between the timing of metamorphosis and water temperatures from mid-March to mid-July illustrate the cumulative effect of temperature on growth and development to metamorphosis. We observed a similar pattern between date of metamorphosis and temperature when only fish collected in July were examined (data not shown); however, there were some differences in the timing of metamorphosis between July-caught and August-caught fish that suggest a potential for post-settlement selection on date of metamorphosis. The potential for selection on the timing of metamorphosis and other early life traits are addressed in Fedewa (2015).

In our study, both annual and individual variation in timing of metamorphosis was strongly related to temperature. While variation in individual temperature conditions (T_{Indv}) explained the majority (>70%) of metamorphosis phenology, annual temperature means ($T_{MetRange}$) across the date range of metamorphosis were also related to the timing of metamorphosis. The annual thermal regime represents the effect of temperature variation experienced by the entire cohort, driving earlier dates of metamorphosis in warmer years and later dates in colder years. The individual temperature metric indicates the seasonal pattern

where individuals metamorphosing earlier in the year are doing so at lower temperatures than fish of the same cohort metamorphosing later in the year.

Another possible factor contributing to the significant correlations between metamorphosis timing and pre-metamorphosis temperatures is that temperature may influence the phenology of earlier life history events such as spawning and hatching. In the absence of strong compensatory mechanisms, a temperature-dependent shift in the time of spawning would result in associated shifts in the time of hatching and metamorphosis. If low temperatures delay spawning activity, the temporal autocorrelation of conditions would result in lower temperatures during the egg and larval stages that may further delay hatch and metamorphosis. The effects of thermal history could then be carried across life history stages, contributing to variation in phenology across years with varying thermal regimes (Dougherty et al., 2007). The timing of metamorphosis and settlement have been associated with temporal variation in spawning in other flatfish studies (Beggs and Nash, 2007; Genner et al., 2010), and a strong relationship between late-larval temperatures and the timing of metamorphosis in NRS suggests that cohorts may display temperature-driven phenological shifts in earlier life stages as well.

The observation of slower than expected growth in 2011 suggests that larval growth may, at least in some years, be restricted by a factor other than temperature, such as food availability. In this study, the timing and magnitude of the spring bloom were used as proxies for overall system productivity. The highly variable and frequently negative correlations between spring bloom dynamics and larval growth metrics suggest that limited availability to food may not be the primary factors driving larval growth dynamics. The NRS life history strategy is an intermediate to "early production" (i.e. winter spawners) and "late production" phenologies (i.e. summer spawners) (Doyle and Mier, 2012). Therefore, NRS early larval stages coincide with

low-production, pre-bloom conditions well before the onset of the spring bloom and peak of chl-*a* concentrations. It is hypothesized that mid-winter to spring spawning species such as NRS could use protracted spawning as a mechanism to compensate for this potential mismatch with food resources (Mertz and Myers, 1994). Interannual variation in phytoplankton production, for example, could be mediated by the protracted spawning window of NRS (~January to April), resulting in a subset of larvae that may be less sensitive to trends in phytoplankton dynamics.

Lack of a strong correlation between spring bloom dynamics and larval growth metrics could also suggest that primary production is not a reliable indicator of NRS prey availability. While little research has been done on NRS larval feeding ecology, walleye pollock, a co-occurring mid-winter spawner, primarily consume copepod eggs, nauplii and copepodites as larvae (Duffy-Anderson et al., 2002). Pollock recruitment in the Bering Sea has been linked to secondary production, with zooplankton production being more closely tied to water temperatures than spring bloom dynamics (Hunt et al., 2002; Hunt et al., 2011). Furthermore, zooplankton production can be out of phase with primary production as overwintering zooplankton and nauplii provide important food resources to larval fish prior to the onset of the spring bloom in the Gulf of Alaska (Napp et al., 1996). Overall, there is currently a lack of information available on the foraging environment of NRS larvae and whether poor foraging conditions could be responsible for episodes of slow growth such as that observed in 2011.

In conclusion, this study provides valuable insight on temperature-driven shifts in the early life history of NRS. Size at hatch and larval growth rates generally increased with water temperatures, but growth outliers indicate the potential for multiple controlling factors. Temperatures in the Gulf of Alaska were the best predictor of variation in the date of metamorphosis with both annual thermal regimes and individual temperature conditions

experienced contributing to plasticity in the timing of metamorphosis. Conversely, NRS metamorphosed across a relatively narrow range of body sizes, suggesting that metamorphosis is a size-dependent process and acts as a point of convergence to reduce variability induced during larval stages. While we have demonstrated the influence of temperature variation on field patterns of growth and metamorphosis in larval NRS, it is not yet clear how temperature influences the timing of earlier life history events such as spawning and hatching as well as describing the carry-over effects between life stages. Determining thermal effects on phenology of these earlier, as well as post-metamorphic stages would clarify the cumulative across-stage influence of environmental conditions and climate change responses in recruitment of NRS.

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Table 1. Sampling dates of age-0 northern rock sole collected in two Kodiak Island nurseries (Holiday Beach, HB and Pillar Creek Cove, PCC) and sample sizes of northern rock sole otoliths with growth metric estimates from microstructural analysis. Sample sizes vary because not all growth metrics were able to be estimated for each individual. '*ND*', or no data, refers to the lack of any readable otoliths during the corresponding period.

Year	Site	Month	Hatch check width (HC_W)	Early larval (EL_{IW})	Larval (L_{IW})	Metamorphic check width (MC_W)	Post-metamorphic (PM_{IW})	Date of metamorphosis (DOM)[1]
2005	HB	19-20 Jul	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
		22 Aug	17	16	24	26	21	21 (0)
	PCC	20 Jul	4	4	5	7	*ND*	*ND*
		23 Aug	20	11	20	27	19	19 (0)
2007	HB	18-19 Jul	4	3	5	8	4	4 (0)
		23, 25 Aug	17	20	25	25	22	22 (1)
	PCC	20 Jul	6	5	8	9	7	7 (3)
		24 Aug	23	22	24	25	23	23 (4)
2009	HB	14 Jul	20	23	26	26	17	17 (7)
		23 Aug	9	9	14	18	9	9 (0)
	PCC	17, 19 Jul	23	24	27	26	22	22 (9)
		24-25 Aug	14	15	20	21	15	15 (3)
2010	HB	14 Jul	18	16	19	22	10	10 (4)
		23-24 Aug	16	15	20	18	19	17 (1)
	PCC	15 Jul	15	18	21	20	14	14 (8)
		25-26 Aug	22	22	23	26	23	22 (0)
2011	HB	16, 20 Jul	11	12	12	13	11	11 (6)
		26 Aug	11	11	11	13	8	8 (0)
	PCC	15, 17 Jul	9	11	13	13	11	11 (4)
		23, 29 Aug	7	6	9	14	11	11 (0)

[1]Sample size represents number of fish for which DOM could be estimated from PM_{IW} and ventral otolith growth (VOG); the number in parentheses is the number of samples with direct estimates of DOM used in the development of the DOM model.

Table 2. Pearson product-moment correlation coefficients *(r)* for comparisons between annual means of northern rock sole larval growth metrics, 15-d mean larval temperatures from the GAK1 buoy (20 m depth) and 8-d mean chlorophyll-*a* concentrations (MODIS-Aqua, 9 km).

Larval growth metric	15-d mean temperatures				
	1 Jan-14 Jan	15 Jan-29 Jan	30 Jan-13 Feb	14 Feb-28 Feb	1 Mar-15 Mar
Mean size at hatch	0.88*	0.89*	0.69	0.86	0.93*
Mean early larval growth	0.50	0.49	0.15	0.33	0.60
Mean larval growth	0.39	0.46	0.00	0.25	0.46
Larval growth metric	**8-d mean chlorophyll-*a* concentrations**				
	10 Feb-17 Feb	18 Feb-25 Feb	26 Feb-5 Mar	6 Mar-13 Mar	14 Mar-21 Mar
Mean size at hatch	-0.23	-0.67	0.00	-0.43	0.75
Mean early larval growth	-0.38	**-0.96***	-0.71	0.02	0.46
Mean larval growth	-0.66	**-0.93***	-0.52	-0.35	0.26

Note: *p < 0.05, **p < 0.01, ***p < 0.001. Critical values were adjusted to account for multiple comparisons (Bonferroni adjustment) and no *p*-values were significant after correction (*p <* 0.01). *n* = 5 for all comparisons.

Table 3. Pearson product-moment correlation coefficients *(r)* for comparisons between annual means of northern rock sole metamorphosis metrics, 15-d mean metamorphosis temperatures from Trident Bay, AK (10 m depth) and spring bloom dynamics. Pre-metamorphosis temperature (Pre_{Met}) was determined as the mean temperature across the 14-d period prior to the onset of metamorphosis each year. Timing of the spring bloom ($Bloom_{Onset}$), magnitude of the bloom ($ChlA_{Max}$) and March-May average chl-*a* concentration ($ChlA_{Spring}$) were determined with 8-d mean chlorophyll-*a* concentrations (MODIS-Aqua, 9 km).

Growth metric	15-d mean temperatures								
	Pre_{Met}	19 Mar-2 Apr	3 Apr-17 Apr	18 Apr-2 May	3 May-17 May	18 May-1 Jun	2 Jun-16 Jun	17 Jun-1 Jul	2 Jul-16 Jul
Mean size at metamorphosis	0.44	0.22	0.13	0.27	0.40	0.74	0.66	0.74	0.86
Mean date of metamorphosis	**-0.99****	-0.95	-0.96	**-0.98****	**-0.97****	-0.87	-0.87	-0.79	-0.64

Growth metric	Spring bloom dynamics		
	$Bloom_{Onset}$	$ChlA_{Max}$	$ChlA_{Spring}$
Mean size at metamorphosis	-0.03	0.33	0.47
Mean date of metamorphosis	0.65	0.38	-0.51

Note: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Critical values were adjusted to account for multiple comparisons (Bonferroni adjustment) and significant values after correction are indicated in **bold.** $n = 5$ for all comparisons.

Table 4. Top five model results to evaluate spring bloom dynamics and temperate effects on individual date of metamorphosis in northern rock sole.

Model	Environmental variables	AIC_c	Δ_i	w_i
M1	T_{Indv}, $T_{MetRange}$	1543.977	0.000	0.4223
M2	T_{Indv}, $T_{MetRange}$, T_{Indv} x $T_{MetRange}$	1545.344	1.3669	0.2132
M3	T_{Indv}, $T_{MetRange}$, $ChlA_{Indv}$, $Bloom_{Onset}$	1545.411	1.4345	0.2061
M4	T_{Indv}, $T_{MetRange}$, $Bloom_{Onset}$	1545.940	1.9627	0.1583
M5	$ChlA_{Indv}$, $ChlA_{PreMet}$, $Bloom_{Onset}$	2256.338	712.361	<0.001

Note: T_{Indv}, mean temperature across 14 d period prior to individual date of metamorphosis estimates; $T_{MetRange}$, annual mean temperature across range of all estimated metamorphic dates; $ChlA_{Indv}$, mean chl-*a* concentration across 14 d period prior to individual date of metamorphosis estimates; $ChlA_{PreMet}$, annual mean chl-*a* concentration across 14 d period prior to the mean estimated date of metamorphosis for each year; $Bloom_{Onset}$, Julian date of spring bloom onset. Akaike information criteria adjusted for small sample sizes (AIC_c), difference between AIC_c of the best model (Δi) and Akaike weight (w_i) are given for each model.

Figure captions

Figure 1. Lab-reared northern rock sole developmental stage and standard length (SL) with corresponding sagittal otolith structure, otolith width (OW) and days-post-hatch (dph): a) Larval stage; bilaterally symmetrical eyes (OW 99.96 µm, SL 10.40 mm, ~58-63 dph); b) Initial eye migration and formation of accessory primordia (OW 225.54 µm, SL 12.39 mm, ~85-90 dph); c) Migrating eye at the dorsal edge of head (OW 193.63 µm, SL 11.49 mm, ~85-90 dph); d) Formation of additional accessory primordia (OW 248.8 µm, SL 12.40 mm, ~85-90 dph); e) Initial metamorphic check formation (OW 308.39 µm, SL 12.92 mm, ~85-90 dph); and f) Dorsal fin reaches the migrating eye, enhanced pigmentation and complete formation of metamorphic check (OW 328.85 µm, Metamorphic check width 276.73 µm, SL 13.98 mm, ~88-93 dph).

Figure 2. Map of Pillar Creek Cove and Holiday Beach field sampling sites off the northeast coast of Kodiak Island, Alaska, USA.

Figure 3. Northern rock sole sagittal otolith landmarks and a) post-metamorphic and b) early larval and larval growth metrics used in the study.

Figure 4. Interannual variability in a) mean GAK-1 temperature records corresponding with northern rock sole larval stages; b) mean Trident Basin temperature records corresponding with northern rock sole metamorphosis; c) mean chl-a concentrations and the annual date of spring bloom onset in waters surrounding Kodiak Island, Alaska. Annual bloom onset dates are indicated by stars.

Figure 5. Annual mean (± 1 SE) of a) hatch check width, b) mean early larval growth and c) mean larval growth of northern rock sole. Letters indicate significant differences from Tukey-Kramer post-hoc analyses (ANOVA, $p < 0.05$).

Figure 6. Influences of temperature on size, growth and phenology of northern rock sole a) Mean size at hatch in relation to mean daily average temperature in February (± 1 SE); b) Mean early larval growth in relation to mean daily average temperature in March (± 1 SE); c) Mean larval growth in relation to mean daily average temperature in March (± 1 SE); d) Mean date of metamorphosis in relation to mean daily average temperature between 18 April and 17 May (± 1 SE). Symbols: 2005, ⊠ ; 2007, ■; 2009, ●; 2010, ▲ ; 2011,◆. Annual sample sizes for each metric shown in Table 1.

Figure 7. Interannual variation in estimated date of metamorphosis of northern rock sole individuals. See text for details on predictive model.

Figure 8. Estimated date of metamorphosis of northern rock sole individuals in relation to temperatures experienced during the two weeks prior to metamorphosis (T_{Indv}). Fitted lines (± SE) represent the temperature range experienced by individuals across each year during metamorphosis.
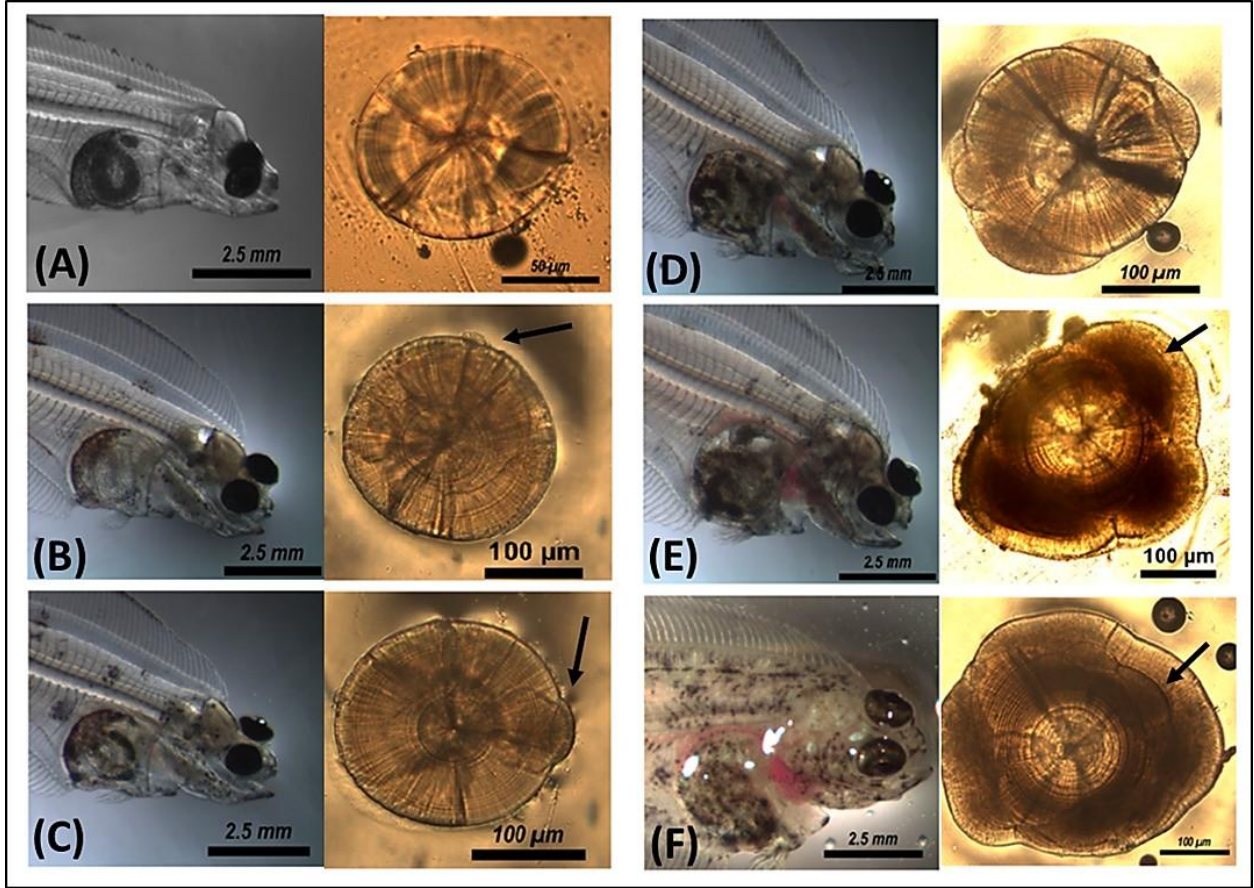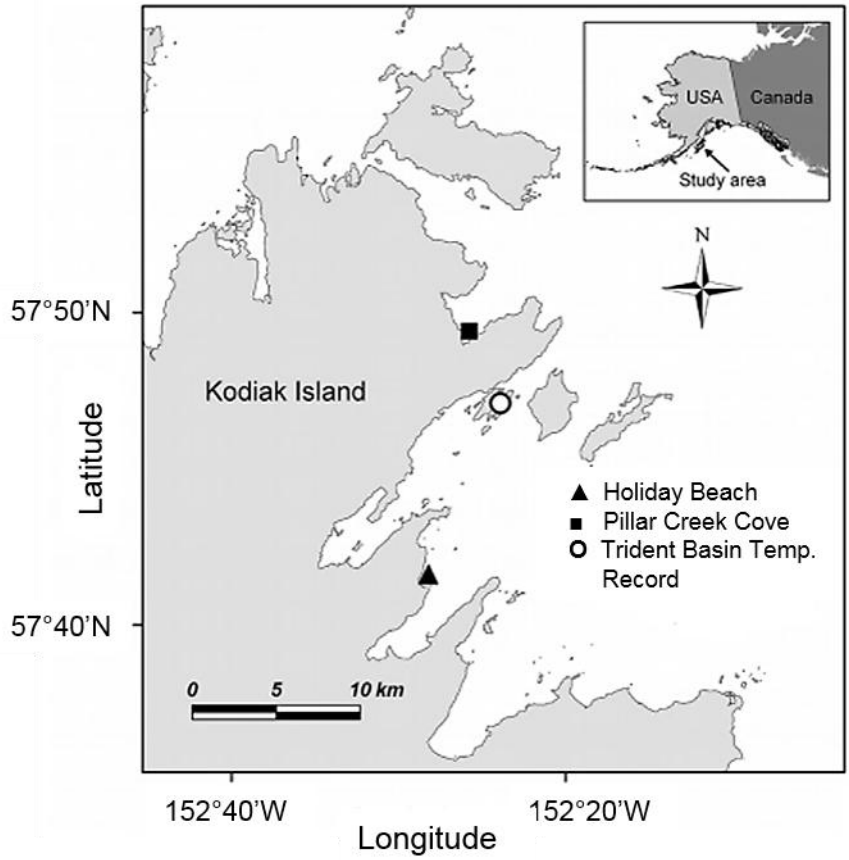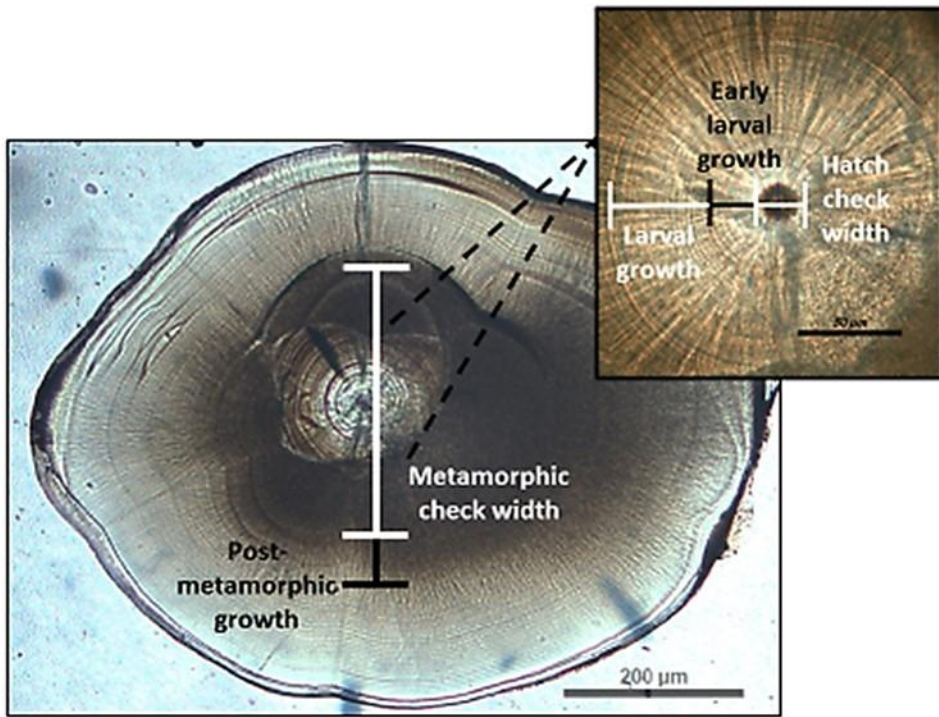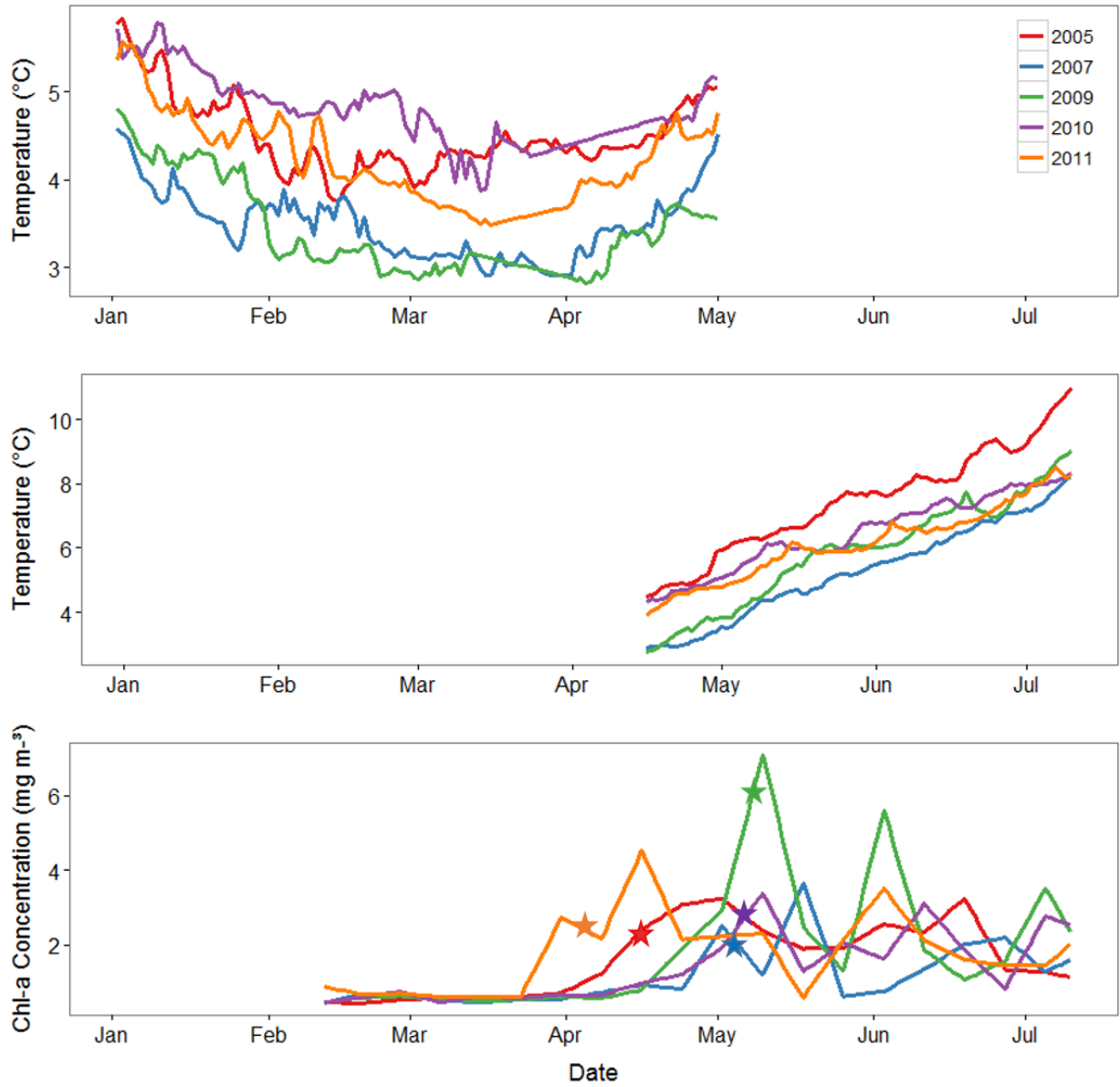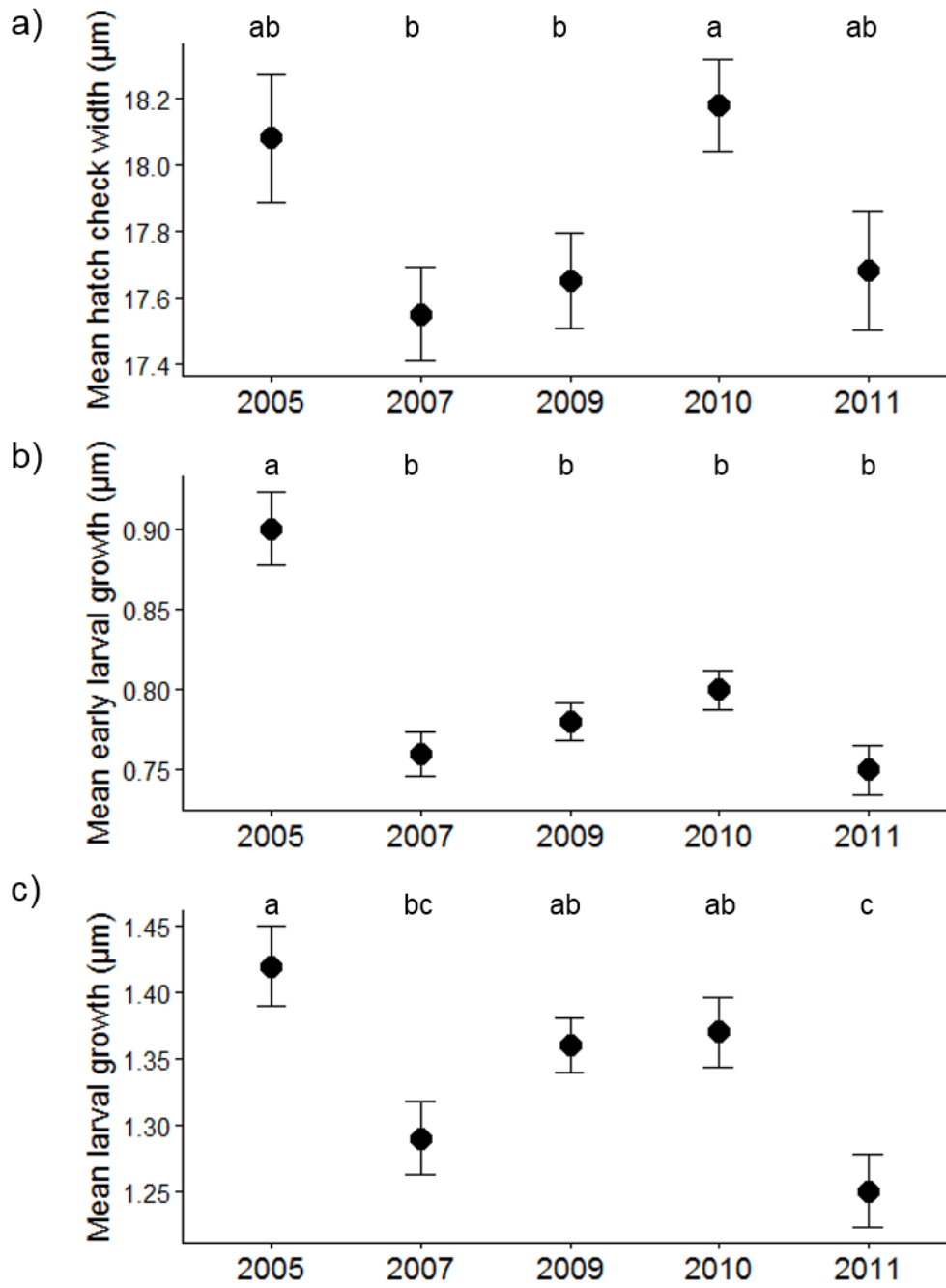
Fig. 1

Fig. 2
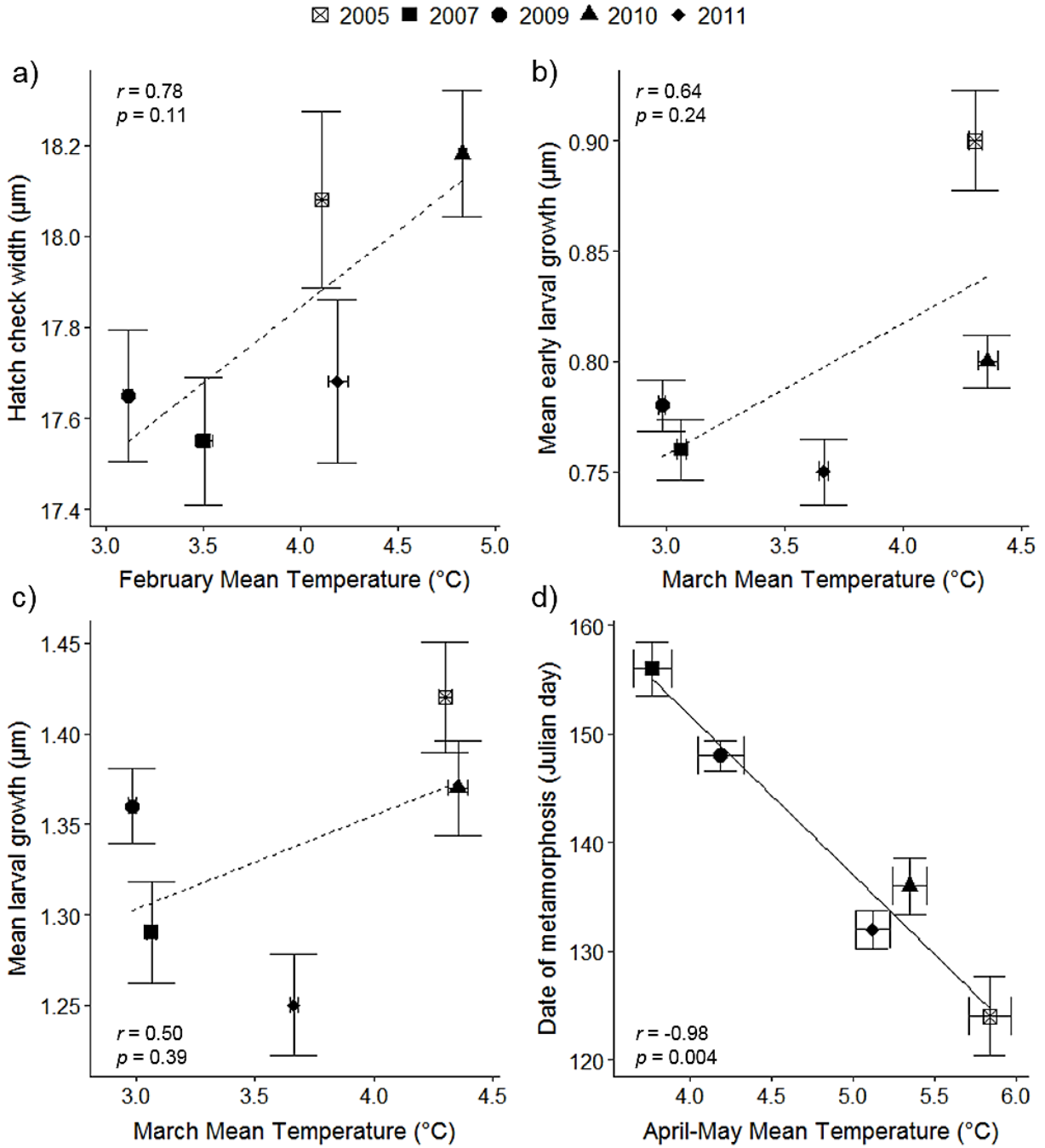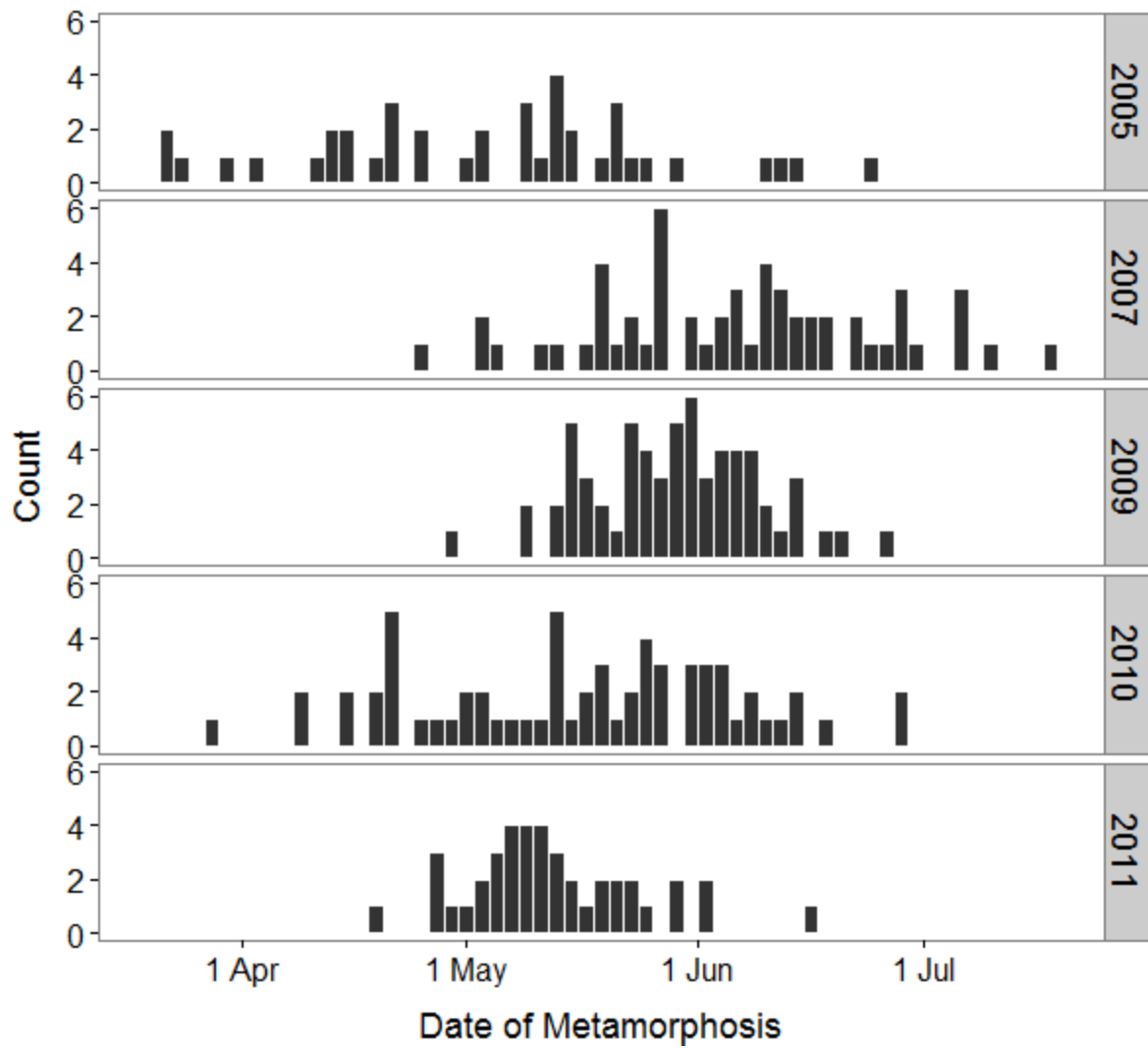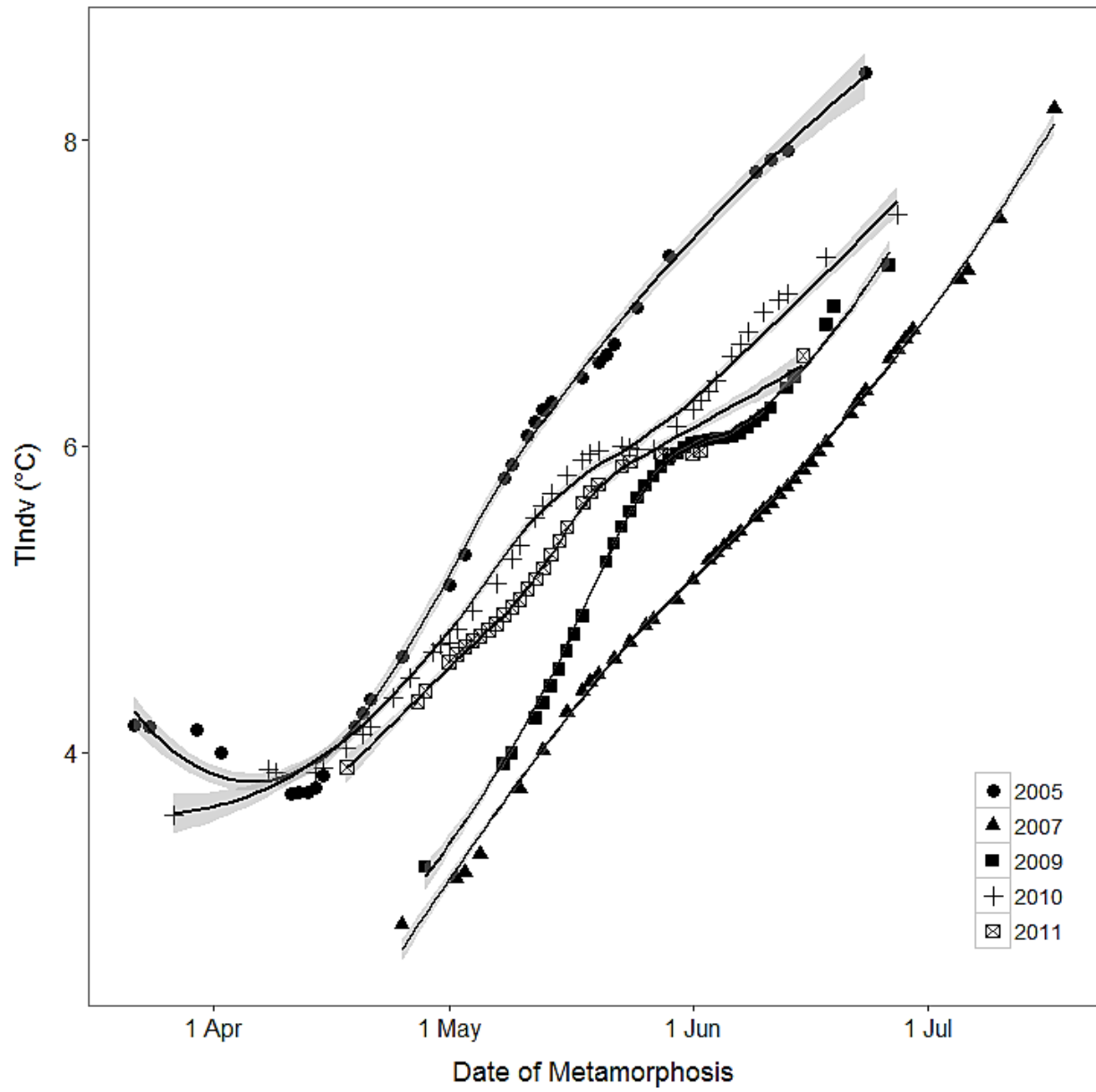
Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Highlights:

- We quantified variation in early life history characteristics of northern rock sole

- Timing of metamorphosis was related to temperature variability

- We observed a constraint in the variation in fish size at metamorphosis

- Early life history characteristics were not related to phytoplankton production