

**ROLE OF TEMPERATURE-MEDIATED EMBRYO DEVELOPMENT IN THE
RANGE EXPANSION OF CUNNER INTO THE BAY OF FUNDY**

by

Krystal Woodard

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Supervisor: Heather Hunt, Ph.D., Biology AND Rémy Rochette, Ph.D., Biology

Examining Board: Ben Speers-Roesch, Ph.D., Biology, Chair

Tim Alderson, Ph.D., Mathematics and Statistics.

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ABSTRACT

Increasing sea-surface temperature has been shown to affect the distribution of coastal fishes. Samples obtained from a monitoring program (2009-2015) of shallow cobble-bottom sites in southwest Bay of Fundy provided the first record (2012) of juvenile cunner *Tautoglabrus adspersus* in the region. The cunner range extends along the North American Atlantic coast to Newfoundland, but only occasional adults were observed in the Bay of Fundy. To determine whether this recent appearance of cunner was related to increased temperature better supporting embryo development and hatch, lab experiments were conducted to complement field observations of juvenile cunner presence/absence and density. Embryos were held in the lab at constant temperatures at 1°C increments ranging from 11-15°C. Hatch was found to increase with temperature, and was markedly greater at temperatures $\geq 13^{\circ}\text{C}$ than at lower temperatures. Relationships observed between temperature and hatch in the lab helped explain variation in density of young-of-the-year cunner among study sites and years. This study provides evidence that increasing water temperature is resulting in range expansion of a small coastal fish into the Bay of Fundy, and that this expansion is at least partly related to increased hatching success.

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List of Symbols, Nomenclature or Abbreviations

AIC	Akaike Information Criterion
ANOVA	analysis of variance
Area	region in which sites were selected for the deployment of bio-collectors
BoF	Bay of Fundy
Dpf	days post fertilization
HPf	hours post fertilization
SD	standard deviation
Site	deployment location of bio-collectors within an area
SR	species richness
SST	sea surface temperature
TL	total length, length of a fish measured from the tip of the snout to the tip of the longer lobe of the caudal fin
Tukey HSD	Tukey honest significant difference
YOY	young-of-the-year

Introduction

The ocean is an important part of the planet's buffering system for solar heat and carbon dioxide (CO₂) emissions, absorbing up to 80% and 50% of these, respectively (Sabine *et al.* 2004, Levitus *et al.* 2000, Domingues *et al.* 2008). With the increase in anthropogenic sources of CO₂, oceans are expected to absorb increasing amounts of this “greenhouse gas” from the atmosphere in the future (Sabine *et al.* 2004). These anthropogenic emissions of CO₂ have been shown to significantly affect oceans, increasing temperature and thereby altering vertical thermal stratification and circulation patterns (EcoAP 2012, Smith *et al.* 2012). Among these changes to oceanographic conditions, changes in water temperatures have been shown to have the largest effect on organisms in marine environments (Hobday and Pecl 2014). Due to thermal preferences, temperature can be a major factor in determining species ranges (Sunday *et al.* 2012). Due to increased sea-surface temperature, marine species might be forced to choose between tolerating suboptimal conditions, typically warmer than their ideal temperature, or to avoid these areas and select instead new locations that were previously too cold (Sunday *et al.* 2012). It has been shown that mobile species such as temperate shrimp (Richards *et al.* 2012) and fishes (Sorte *et al.* 2010) are vulnerable to the negative physiological and population effects of increased ocean temperature (Hobday and Pecl 2014).

Between 1950 and 1999, ocean temperature has increased by a mean of 0.5°C per decade, but not all ocean regions are warming at the same rate. Over this 50-year period, 24 ocean areas have been identified as “hotspots”, warming at the highest rate

globally (Hobday and Pecl 2014, Pecl *et al.* 2011). A hotspot of particular interest is the Gulf of Maine, including the Bay of Fundy (BoF), which is warming 90% faster than the mean global warming rate (Rayner *et al.* 2003, Wu *et al.* 2012, Hobday and Pecl, 2014). As a result of this marked thermal increase, the Gulf of Maine ecosystem has been changing, with temperature sensitive species particularly affected (Rayner *et al.* 2003). Temperate species of shrimp, for example, are sensitive to changes in temperature, where colder temperatures lead to increased larval recruitment and warmer temperatures could significantly and negatively affect populations (Richards *et al.* 2012). Such effects have been observed within the shrimp *Pandalus borealis* fishery in the Gulf of Maine, where increases in water temperatures have led to a decrease in shrimp landings (Richards *et al.* 2012). The Gulf of Maine Atlantic cod (*Gadus mohura*) fishery has also been affected, with increasing temperatures seemingly causing a decrease in spawning stock biomass (Pershing *et al.* 2015). This decrease in cod abundance in the Gulf of Maine was probably caused by increasing water temperatures that increased metabolic cost and decreased weight at age, resulting in lower probability of surviving adults (Pershing *et al.* 2015, Deutch *et al.* 2015) and overfishing of this smaller population (Pershing *et al.* 2015).

Effects of increasing water temperature are species specific, and can have far-reaching impacts on a population (Walther *et al.* 2002, EcoAP 2012), including changes in recruitment, distribution, and mortality rates (Yemane *et al.* 2014). Many studies examining the biological impacts of climate change in the marine environment have focused on shifts in distribution of species (Sorte *et al.* 2010), including fish species, through range expansion or contraction, as species shift to maintain themselves in areas

where temperature is suitable for different physiological/biological process (Yemane et al. 2014). Recent studies have shown that an increasing number of warm-water fishes are expanding their range and becoming more abundant pole-ward (Sorte *et al.* 2010). Over the last ten years, nearly 75% of reported changes in fish species distributions have been in a pole-ward direction, as would be predicted in response to increasing water temperatures (Root *et al.* 2003, Chen & Wilson, 2002; Sunday *et al.* 2012). For example, in the North Sea in the Eastern Atlantic, fish species endemic to warmer southern waters, including the red mullet *Mullus surmuletus* and the sardine *Sardina pilchardus*, have begun to establish themselves in cooler northern waters, resulting in a maximum range expansion of 7 degrees of latitude (Beare *et al.* 2004, ICES 2006). From 1981 to 1995, zebra perch *Hermosilla azurea* along the Eastern Pacific, off the U.S.A expanded their range 440 km northward along the coast from 1981 to 1995, which has been associated with warming that forced adults to travel into areas with an optimal thermal regime (Sturm and Horn 2001). Zebra perch is not the only species along the Western USA coast that has shown signs of range expansion; adult squid *Dosidicus gigas* have expanded their range ~1595 km northward from 1997-2005, a mean rate of ~200 km per year, and are now reproducing in new habitats (Brodeur *et al.* 2006). This range expansion has been mainly attributed to increasing sea-surface temperatures causing increased larvae survival rates in “northern areas” (Brodeur *et al.* 2006). If waters continue to warm at the proposed rate, this trend is expected to continue, especially in global warming hotspots, such as the Gulf of Maine (Hobday and Pecl 2014). There have indeed been cases of range expansion and distribution shifts in the Gulf of Maine, such as black striped bass *Centropristis striata* moving northward to

Midcoast Maine (Bell *et al.* 2015), yet surprisingly, there are currently no reports of warmer-water fish species moving northwards into the BoF.

A new sampling program in the Quoddy region of the BoF, the northernmost portion of the Gulf of Maine, has recently provided the first records of juvenile cunner *Tautoglabrus adspersus* in the region. The cunner is a temperate reef fish, typically found in shallow (<8 m) rocky bottom habitats (Green 1975) on the coast of the Northwest Atlantic (Collette & Klein-MacPhee 2002; Ojeda and Dearborn 1991). The cunner is a wrasse (Labridae) and the most northern member of the family to be found in the Northwestern Atlantic. Currently, cunner are found along the Atlantic coast of North America from Chesapeake Bay, Maryland to Conception Bay, Newfoundland (Collette & Klein-MacPhee 2002). There are regions within this range, such as the New Brunswick coast of the BoF, where they are scarce, and only large individuals are occasionally observed (Collette & Klein-MacPhee 2002). The absence of young cunner in the BoF may be related to temperature. The BoF has relatively low summer water temperatures, as its large tides cause considerable vertical mixing and reduce stratification and warming of surface waters (Wahle *et al.* 2013). Cunner spawn during summer, and have buoyant eggs that are typically found in the upper 5 m of the water column (Kuntz and Radcliffe 1917, Williams 1968). Ichthyoplankton tows have found cunner embryos in waters ranging from 10 to 26°C (Wheatland 1956, Williams 1968), but it has been suggested that the optimal thermal range for cunner spawning is >13°C (Collette & Klein-MacPhee 2002). Very little is known about the relationship of cunner embryonic development to temperature. The scarcity of cunner in the BoF, and

especially of young individuals, has been hypothesized (Colette & Klein-MacPhee *et al.* 2002) to result from the inability of their embryos to develop in the cold summer water temperatures of the BoF, as summer sea surface temperatures have historically been $<13^{\circ}\text{C}$ (Reid, 1929, Collette & Klein-MacPhee 2002, Mills *et al.* 2013). However, it is possible that increasing temperature may enable cunner, a temperature sensitive species that is primarily found in relatively warmer summer water temperatures (Green and Farwell 1971, Guthjar-Gobell *et al.* 2002), to establish permanent populations in the BoF.

This project has two aims: (1) to confirm and document the recent appearance, first observed in 2012, of young juvenile cunner in the Quoddy region of the Southern BoF, and (2) to investigate if this expansion in distribution of cunner from the Gulf of Maine into the BoF can be linked to increased sea-surface temperature allowing for more successful embryo development. First, the occurrence and abundance of cunner in the Quoddy region was documented from 2009-2015 using data from cobble-filled bio-collectors that have been deployed in this region since 2007. Second, sea-surface temperature in the same region over the past 10 years was examined to determine whether increases in temperature coincide with changes in the presence of juvenile cunner in the bio-collectors. I used a laboratory experiment to test the effect of temperature on the development of cunner embryos. Finally, results from the lab experiment were used to determine whether the density of juveniles in bio-collectors in the BoF could be predicted from the relationship between temperature and embryo hatch. I hypothesized that the range of cunner is expanding into the BoF due to

increasing water temperature that is enhancing embryo development and hatch in the BoF.

Methods

Description and deployment of bio-collectors

Passive benthic cobble-filled bio-collectors (henceforth referred to as bio-collectors) have been used to monitor lobster settlement (Wahle *et al.* 2009) and biodiversity of fish and decapod crustaceans (Hunt *et al.* 2017) in various areas of Atlantic Canada and the Northeast USA. The cobbles inside the bio-collectors imitate a rocky habitat and serve to attract various species that seek shelter inside the heterogeneous matrix they provide. The bio-collectors measure 61 cm x 91.5 cm x 15 cm (width x length x height) and weigh approximately 80 to 115 kg when filled with cobble (7-17 cm in diameter). The frame of the bio-collectors is made of 10-gauge vinyl-coated wire, with a 37mm mesh size. The bottom of the bio-collectors is lined with a plastic screen with 1 mm PetScreen™ mesh to reduce loss of organisms on retrieval. The bio-collectors were deployed for approximately four months, from July to October each year, from 2009 to 2015, within the Quoddy region of the Southern BoF. They were deployed at 3-6 sites within each of six areas: Beaver Harbour, Deadman's Harbour, Maces Bay, Seeley's Cove, Passamaquoddy Bay and Deer Island (Figure 1). On average 21 bio-collectors, with a line and buoy attached to allow for retrieval, were deployed at each site in different years on cobble bottom at 5-10 m depth below mean low water. The number of bio-collectors deployed each year varied among areas, depending on the needs of different projects (Table 1). Bottom temperature was

measured by placing one HOBO Water Temperature Pro v2 data logger on two bio-collectors per site, with temperature recorded every 30 min for the duration of deployment. After the bio-collectors were retrieved, all larger species, such as fish and decapods, were removed and preserved by freezing or by placing in ethanol, except for 2012 when only a subsample of the collected fish was preserved. In all other years, each fish was identified, and its total length (TL) measured.

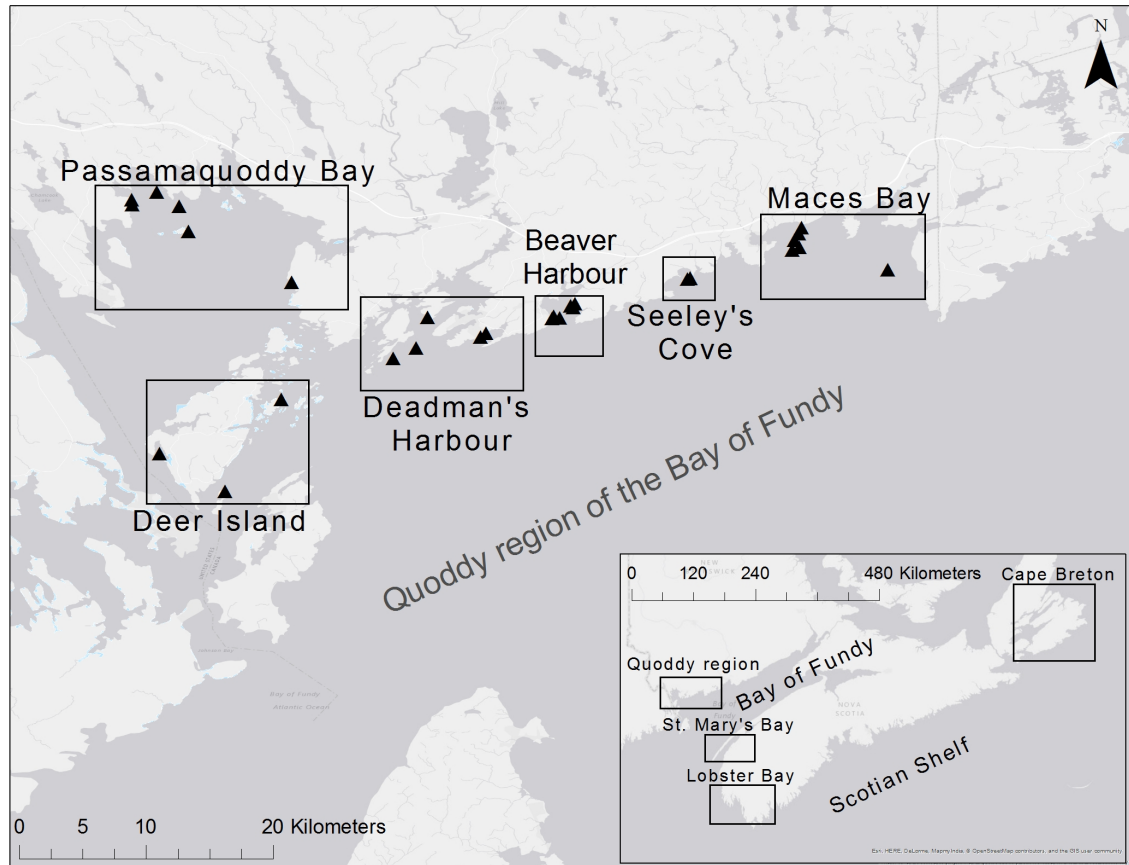


Figure 1: Map of sites (triangles) within the areas (boxes) where bio-collectors were deployed within the Quoddy region of the Southern Bay of Fundy (main map) and along the Scotian Shelf in Nova Scotia (insert). Cunner from the three areas within the Scotian shelf (St. Mary's Bay, Lobster Bay, Cape Breton) were used to approximate the total length (TL) of young-of-the-year (YOY) cunner, which was then used to assign cunner found in the Quoddy region of the Southern BoF as YOY or older (see *Methods: Determining the size of YOY cunner*).

Table 1: Number of bio-collectors deployed and retrieved within the sampling sites and areas of the Quoddy region of the Bay of Fundy, along with the coordinates of each of these sites. “NA” indicates bio-collectors were not deployed in that particular site and year

Area	Site	Longitude	Latitude	Number of retrieved/deployed bio-collectors						
				2009	2010	2011	2012	2013	2014	2015
Beaver Harbour	Centre	-66.720517	45.069883	NA	NA	25/25	19/20	22/22	NA	16/20
Beaver Harbour	East	-66.718483	45.068517	20/20	27/28	27/27	19/20	21/21	16/18	NA
Beaver Harbour	East Sand	-66.717433	45.071350	18/18	NA	NA	NA	NA	NA	NA
Beaver Harbour	Lighthouse	-66.732533	45.062317	18/20	28/30	20/20	18/20	21/21	16/17	17/20
Beaver Harbour	Lighthouse Mid	-66.733683	45.060717	NA	28/30	NA	NA	NA	NA	NA
Beaver Harbour	Southwest	-66.728364	45.061097	18/21	35/35	25/25	NA	NA	NA	NA
Deadman's Harbour	DHI	-66.780767	45.050350	NA	19/20	25/25	20/20	18/18	24/24	26/30
Deadman's Harbour	DHII	-66.784450	45.047750	NA	NA	17/17	18/20	16/18	NA	NA
Deadman's Harbour	Howard Island	-66.830200	45.039850	NA	NA	NA	NA	NA	NA	17/20
Deadman's Harbour	Limekiln	-66.822167	45.061733	NA	NA	NA	NA	NA	NA	18/20
Deadman's Harbour	Man-O-War	-66.846367	45.032483	NA	NA	NA	NA	NA	NA	19/20
Deer Island	Boone Cove/ Fish Island	-66.925752	45.003495	NA	NA	NA	NA	NA	NA	17/20
Deer Island	Doctor's Cove	-66.965559	44.938600	NA	NA	NA	NA	NA	NA	18/20
Deer Island	Fairhaven	-66.992836	44.965200	NA	NA	NA	NA	NA	NA	17/20
Maces Bay	Mud Centre	-66.558800	45.121100	NA	NA	NA	100/100	NA	NA	NA
Maces Bay	Mud North	-66.556567	45.125017	NA	NA	NA	41/42	NA	NA	NA
Maces Bay	Mud South	-66.561833	45.116317	NA	NA	NA	43/43	NA	NA	NA
Maces Bay	No Name Rock	-66.558433	45.111333	NA	20/20	25/25	20/20	19/20	16/17	14/20
Maces Bay	North Welch's Cove	-66.495533	45.095450	NA	NA	NA	NA	NA	NA	16/20
Maces Bay	Pocologan	-66.563417	45.109450	NA	20/20	25/25	20/20	20/20	17/17	18/20
Maces Bay	Welch's Cove	-66.635900	45.089217	NA	NA	NA	NA	NA	NA	20/20
Passamaquoddy Bay	Birch Cove	-67.031750	45.145116	18/20	15/15	25/25	20/20	20/20	15/18	17/20
Passamaquoddy Bay	Birch Cove Mud	-67.031583	45.141633	20/20	NA	NA	NA	NA	NA	NA
Passamaquoddy Bay	Dick's Island	-66.998233	45.140200	20/20	NA	25/25	NA	NA	NA	NA
Passamaquoddy Bay	Hardwood Island	-66.991600	45.122483	NA	NA	NA	NA	17/17	17/18	20/20
Passamaquoddy Bay	Mascarene	-66.918533	45.086316	NA	NA	NA	NA	15/17	18/18	NA
Passamaquoddy Bay	Seal Rock	-67.013933	45.150333	20/20	NA	NA	NA	NA	NA	NA
Seeley's Cove	Seeley's Basin	-66.635817	45.089483	NA	20/20	25/25	NA	20/20	16/17	17/20
Seeley's Cove	Seeley's Cove	-66.637750	45.088650	NA	20/20	25/25	20/20	20/20	17/17	18/20

Determining the size of YOY cunner

In order to determine whether cunner captured in the bio-collectors deployed in the Quoddy region of the Southern BoF were young-of-the-year (YOY) or older individuals, size frequency distributions of total length (TL) were analyzed. These analyses were based on cunner data sets from bio-collectors deployed at sites in Nova Scotia (Lobster Bay, Cape Breton and St. Mary's Bay) from 2007 to 2014 (Fig. 1) deployed at a similar depth and period as the bio-collectors in the Quoddy region of the Southern BoF (Hunt *et al.* 2017). Cunner data from Nova Scotia bio-collectors were selected because those deployed in the Quoddy region did not provide sufficient numbers of individuals for the analyses (normal mixture distribution model requires more than 20 individuals per analysis, i.e. site year combinations to satisfy assumptions). If TL of YOY depends on summer temperature, it is expected that the Nova Scotia samples will allow me to predict the size of YOY cunner in the Quoddy region given the marked overlap in bottom temperature in the two regions (NS: $15.08 \pm 1.11^{\circ}\text{C}$; NB: $12.17 \pm 0.8^{\circ}\text{C}$). Each individual data set (site/year combination) of cunner lengths that met the criteria of having twenty or more individuals was fit with a normal mixture distribution model using the mixtools package in R. This package identifies the peaks of curves in the size frequency data, which are interpreted to represent different age classes; it also provides the median (and standard deviation) TL of the peaks (an age class). This method assumes that each age class is represented by only one peak, and therefore that spawning of the whole population occurs within approximately the same time frame. For all site/year combinations used, the first mode represented the TL of YOY cunner, considering the reported size at hatch (Johansen

1925, Serchuk & Cole 1974, Tupper and Boutilier 1997), and growth rates of YOY cunner (30-79 mm within first year in Massachussetts, Serchuk & Cole 1974; 0.47 mm d^{-1} in Nova Scotia, Tupper and Boutilier 1997). We added the standard deviation (SD) to the median TL of the YOY mode to assign the upper size limit of YOY cunner from a particular site and year.

To determine if the size of YOY cunner varied among sites in relation to bottom temperature, estimates of YOY median length + 1SD in each Nova Scotia site and year combination were regressed against the bottom temperature (HOBO temperature loggers) in each of these site-year combinations for the same time period when the bio-collectors were in the water (7 July to 14 October), which includes the period of early cunner growth following recruitment to the bottom (Reid 1929, Pottle and Green 1979, Faber 1976). Because this analysis indicated no relationship between YOY cunner length and temperature in Nova Scotia (see Results: Determining age of YOY cunner), the cut off value I used to assign BoF cunner as YOY was the mean of the values of median + 1SD TL obtained in the Nova Scotia samples (i.e., 66 mm TL; varied between 33-89 mm across sites). When this analysis was instead based on pooling samples from all Nova Scotia sites, it yielded a somewhat larger cut off for YOY cunner (74.4 mm). The cut off derived from analyzing each site/year combination separately was used to age cunner collected in the Quoddy region of the Southern BoF. However, the same general conclusions are reached whether the YOY size cut-off estimate was based on separate or pooled samples.

Change in cunner presence/ density over time and temperature in the Quoddy region of the Bay of Fundy from 2009-2015

To determine whether the recent observations of young juvenile cunner in the BoF can be attributed to an increase in summer water temperature in the region, cunner presence in the bio-collectors between 2009 and 2015 was related to bottom temperature measured by the HOBO data loggers attached to the bio-collectors. Specifically, daily bottom temperature was averaged from 2 loggers per area from 7 July to 15 September of each year (Fig.1), which approximates the spawning and benthic recruitment period of cunner (Collette & Klein-MacPhee 2002). While sea-surface temperature would have been preferable to bottom temperature to address my study hypothesis, because cunner embryos are pelagic and therefore exposed to surface water temperatures during development, accurate satellite sea-surface temperatures (SST) could not be obtained for many areas, which were very close to shore, and in some cases in small enclosed bays (Fig. 1). In contrast, my bottom temperature data is the result of direct measurements made at the precise locations where cunner were sampled from. Due to strong tidal mixing, temperature at the mouth of the BoF does not strongly stratify with depth during the summer, resulting in temperatures at a depth of 5-10 m, where the bio-collectors were deployed, being similar to those at the surface (Wahle *et al.* 2013). I will therefore use measured bottom temperature as a proxy for SST in my study areas.

Presence/absence data of YOY cunner for each of my study areas (n=6) and years (n=7) in the Quoddy region of the Southern BoF were compared to mean bottom temperature in each area-year using logistic regression. Also, the relationship between the mean density of YOY cunner in the bio-collectors in an area and year, and the bottom

temperature in the same area and year, was investigated with linear, exponential and intercept only models, with AIC used to select the best model. All analyses were completed using the R statistical package version 3.2.2.

Rate of embryo development at varying temperatures

In order to assess the development of cunner embryo (terminology based on Balon 1975) at different temperatures, including potentially identifying a temperature threshold for embryo development, laboratory experiments were conducted using cunner embryos obtained from Kelly Cove Salmon Ltd., who established a cunner hatchery in 2011, at the Huntsman Marine Science Centre, Saint Andrews, NB to use cunner to combat sea lice infestations on salmon. Embryos were first successfully produced by the hatchery in 2013. Historically, wrasses have been used successfully as cleaner fish in salmon aquaculture in order to combat sea lice infestations. Species such as Corking wrasse (*Symphodus melops*) and Ballan wrasse (*Labrus bergylta*) have been used in European countries. In Canada, cunner are currently being investigated for their potential as cleaner fish in aquaculture (MacKinnon 1995, MacKinnon 1997, Gonzalez and deBoer, 2017). At the Huntsman, cunner were bred in large tanks (~1000 L) with approximately 40 breeding-age individuals. At the outflow of the breeding tank, a 200 µm Nitex bag was used to sieve out the embryos. This bag was emptied daily at approximately 8 a.m. The embryos were no older than 24 hours post fertilization (hpf), typically at the blastula stage, and were transported in a cooler filled with seawater from Saint Andrews to Saint John NB.

After arrival in the laboratory at UNB Saint John, the embryos were sterilized in a mixture of 30 L of seawater and 5 mL of 10% formalin to kill any pathogens or parasites that could influence development and hatching success. In a preliminary experiment in which the embryos were not sterilized, 0 out of 9750 embryos successfully hatched.

Two experiments were conducted to test the effect of temperature on cunner embryo development, the first to estimate the timing of hatch and the second to estimate hatching success under different rearing conditions and temperatures. Embryos were held in 30 L Aquabiotek® tanks with chilling and heating capabilities, and exposed to one of five temperatures (11, 12, 13, 14, 15°C), with three replicate tanks per temperature. The room that housed the tanks was maintained at 13°C, to assist in sustaining a constant tank temperature, and it was confirmed via Hobo temperature loggers that each tank maintained its constant set temperature $\pm 0.1^\circ\text{C}$ throughout the experiment.

In the first experiment, embryos were sampled very frequently (see next paragraph for time intervals) to determine the precise time of hatch at the different temperatures. In the second experiment, embryos were sampled less frequently, and two different types of containers were used to hold the embryos to compare hatch success of different rearing conditions. Each experiment used approximately 30 ml of embryos, with ~ 400 embryos ml^{-1} . In the first experiment, 50 embryos were placed into each of 195 petri dishes (5 cm diameter) with holes (38 mm) in the lid and bottom covered with 250 μm Nitex to allow for the exchange of water. 13 dishes were allocated to each of 15 tanks (5 temperatures x 3 tanks/temperature). In the second experiment, 50 embryos

were placed into the petri dishes described for the first experiment (n=5 per tank) and, in addition, 100 embryos were placed into each of 9 plastic beakers (5 cm diameter x 7.5 cm height) filled with 150 mL of seawater (25-28 ppt), 3 per each tank. The beakers were floated on the surface of the water of each tank, so they would be at the same temperature as the water in the tank, by placing them into spaces cut into a 9 cm x 9 cm piece of Styrofoam. In contrast to the petri dishes, no exchange of water occurred within the beakers and water was not aerated. Because similar still-water conditions have been previously used successfully for cunner embryos (Guthjar-Gobell *et al.* 2002), hypoxia was not a concern.

During both experiments, embryos were categorized as follows (Balon 1975) (Fig. 2):

- Pre-hatch: embryo development still occurring; embryo was clear and cell division and development could be seen within the embryo
- Dead: embryo development had stopped; embryo was yellow or opaque in coloring
- Hatched: embryo development was complete and a eleutheroembryo (yolk-fed embryo free from the egg casing (Balon 1975)) was present

In the first experiment, sampling was done every 12h at first, and then every 6h from the time a first larva was observed and until no hatch was observed for 2 successive sampling periods in each temperature treatment. At each sampling period, one dish in each tank was arbitrarily selected, removed from the tank, and placed in a glass bowl filled with seawater, and all embryos were carefully assessed for developmental stage under a dissecting microscope at 1.6x magnification. If the dish had

already been selected, it was placed back into the tank, and another dish was chosen until one that had yet to be sampled from was selected. The petri dish was then placed back into the same tank it was removed from. Once all dishes had been selected once, they were then eligible to be reselected. When a dish was sampled a second time, it was terminated by placing all embryos into a 20mL scintillation vial filled with glycerine-ethanol solution (35%-65%). Assessing the development stage of all embryos in a single dish took less than four minutes.

In the second experiment, samples were taken from petri dishes and beakers only at three times, which were determined based on data obtained from the first experiment: 1- the time at which the first larva was observed at all temperatures, 2- the time at which the greatest number of hatched eleutheroembryos was recorded, and 3- the time at which the greatest number of dead embryos was observed (see Results for more detailed information). Each sample was removed only once from its tank, at which point all embryos were observed and then euthanized, being placed into a 20mL scintillation vial filled with glycerine-ethanol solution (35%-65%). Embryos were removed from dishes with a 1 mL pipette, while embryos in beakers were first filtered out using 250 μ m Nitex and transferred to a glass dish, and then removed using a 1 mL pipette.

A nested two-way ANOVA was performed to compare the proportion of embryos that hatched between the two different rearing treatments (dishes or beakers) and five temperatures; for this analysis, each of the three time periods were analyzed separately. Because hatching success was considerably higher in the beakers than in the petri dishes, subsequent analyses focused only on the data from the beakers. A nested two-way ANOVA was carried out to compare the proportion of hatch of cunner

embryos among rearing temperatures and time (80, 120 and 160 hpf), where rearing tank was a random factor nested within temperature, which was a fixed factor. A Tukey HSD test was carried out as a posthoc test to compare the different combinations of tank temperature and time levels. All statistical analyses were carried out using R version 3.2.2.

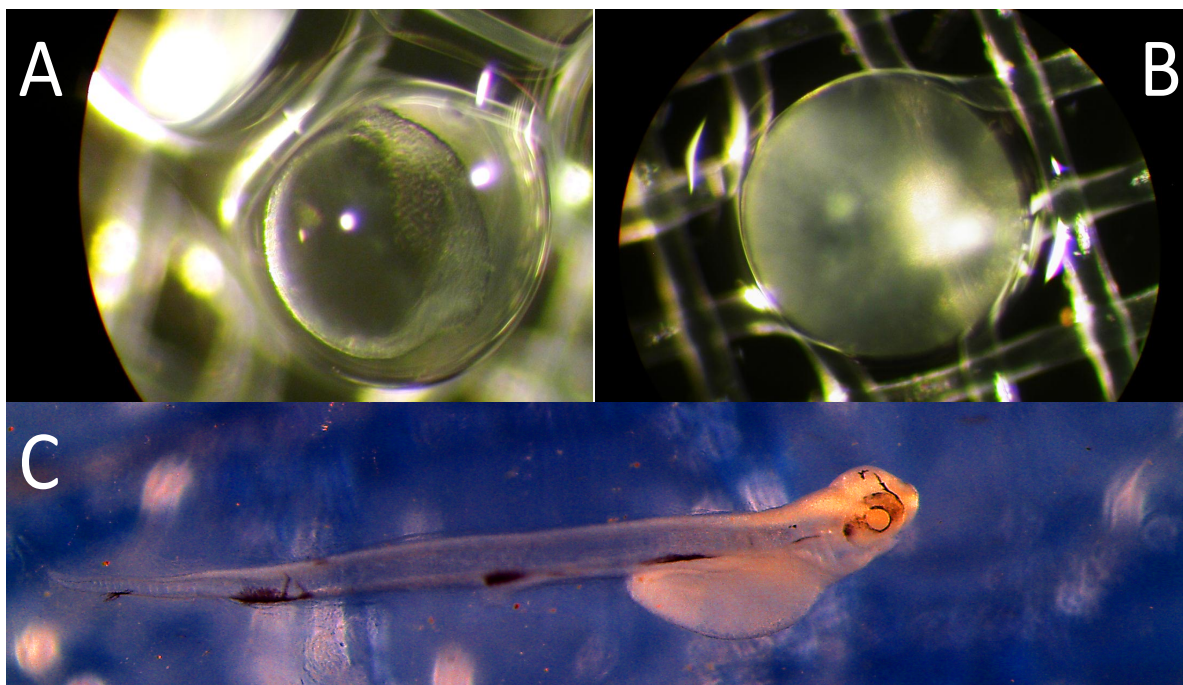


Figure 2: Embryonic stages used to categorize each embryo during a sampling period: (A) pre-hatch, (B) dead and (C) hatched. Photos were taken at 80x using a camera mounted on a Leica S8AP0 microscope. Embryo diameter was approximately 0.5 mm (A&B) and eleutheroembryo measured approximately 1.25 mm TL at 160 hpf(C).

Comparison of field observations and laboratory experiment

To test the hypothesis that the occurrence of young cunner in the Quoddy Region of the Bay of Fundy is related to presence of temperatures required for successful embryo development, I examined whether the effect of temperature on cunner hatch success in the lab helped explain variation in the occurrence of YOY cunner in the bio-collectors in different years and areas. First, I modelled the relationship between temperature and the proportion of embryos that successfully hatched in the second laboratory experiment using an exponential function, a linear regression and an intercept (null) model, and then used AIC to select the best of these three models. These three models were selected based on other studies that investigated relationship between embryo development and temperature, which used either an exponential/ logarithmic or a linear model (Pepin 1991). The null model was tested to determine whether the model selected is better than a model with no relationship to temperature. The best model was then used to predict hatch success in my 6 study areas and 7 years based on the daily mean bottom temperatures recorded in these areas-years over the expected period of larval development and early benthic recruitment (7 July to 15 September). This predicted hatch success was then compared to the density of YOY cunner found in the bio-collectors in each area-year combination, and this relationship was modelled as an exponential function (+ 1 added to density value to remove zeros from the data set), a linear regression, and as in intercept only (null) model, with AIC being used to select the best model.

Results

Determining the size of YOY cunner

No significant relationship was found ($R^2=0.051$, $F_{1,37}=1.404$, $P=0.168$) between the median (+1 SD) TL of YOY cunner sampled from bio-collectors in different sites and years in Nova Scotia and the mean bottom temperature in these sites/years during the expected embryonic and benthic growth period (Fig. 3). Consequently, the maximum size of YOY cunner was found to be the mean median length (+ 1 SD) of YOY cunner across all sites and years in Nova Scotia, 66 mm. This value of 66.0 mm was then used as a size cut-off in assigning individuals found in the Bay of Fundy bio-collectors as YOY (≤ 66.0 mm) or older (> 66.0 mm). Hence, in my study all individuals ≤ 66.0 mm were deemed to be YOY that had hatched and settled during the same summer that they were caught in the bio-collectors.

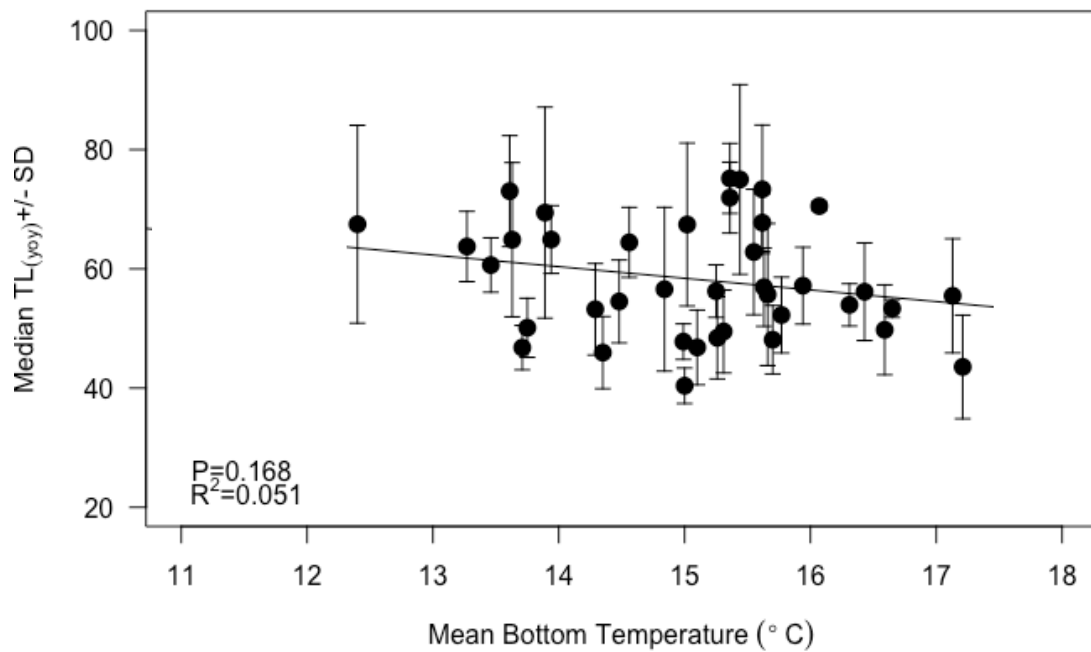


Figure 3: Relationship between median (± 1 SD) total length (TL) of YOY cunner (*Tautoglabrus adspersus*) in cobble-filled bio-collectors deployed in 4 sites on the Scotian Shelf in Nova Scotia from 2007 to 2014 ($n=38$) (Canso, Cape Breton, Lobster Bay and St. Mary's Bay), and mean bottom temperature ($^{\circ}\text{C}$) over the time the bio-collectors were deployed (17 July to 15 September). The black line represents a non-significant linear regression ($F_{1,37}=1.404$, $P=0.168$).

Change in cunner presence/density in the Quoddy region of the Bay of Fundy between 2009 to 2015

No cunner were collected in Beaver Harbour in the Bay of Fundy in 2007 (31 bio-collectors) and 2008 (49 bio-collectors) (Hunt and Rochette unpubl. data). Similarly, no YOY cunner was found in a total of 687 bio-collectors deployed in any areas in the southwestern Bay of Fundy from 2009 to 2011 (Fig. 4). The first occurrence of cunner in bio-collectors in the Quoddy region was in 2012, but only a subsample of fish were preserved that year, and density and locations in which fish species occurred was not recorded. In 2013, cunner were found in bio-collectors at 5 of 6 areas (none in Deadman's Harbour), with density being highest in Beaver Harbour (Fig. 4). The following year, in 2014, YOY cunner (and now also some ≥ 1 year old cunner) were again present in the bio-collectors, but densities were markedly lower in all locations compared to 2013, especially in Beaver Harbour (Fig. 4). In 2015, cunner continued to be present at low densities, but YOY were only found in Passamaquoddy Bay. Over the 6 years of bio-collector deployment, no YOY cunner were found in bio-collectors at Deadman's Harbour. However, ≥ 1 year old cunner were found in bio-collectors in this area in 2013.

When examining the total length of cunner sampled in the Quoddy region of the Bay of Fundy from 2012 to 2015, a shift can be seen (Fig. 5). In 2012, the first year cunner were collected, the majority of the cunner can be deemed YOY based on their total lengths (< 66 mm). Unlike those first samples, in 2015 all but three cunner found were greater than one year of age, and these included some of the largest cunner that were found in the bio-collectors during my study, with total length of some individuals

exceeding 135 mm (Fig. 5). The 66mm cut off derived from the NS data is near the end of the first mode in the 2013 BoF data (~48-70 mm), indicative of the first year class, and before the second mode (~68-90 mm), which likely represents the second year class.

The size cut-off used for YOY based on the NS data (74.4 mm) is very similar to the size cut off I would have obtained based on the length-frequency distribution of cunner sampled in the Quoddy region in 2013 (74.0 mm), which is the year with the greatest number of cunner.

Relationship between temperature and cunner presence/absence and density in the Quoddy region of the Bay of Fundy from 2009 to 2015

Mean summer bottom temperature (daily mean between July 7th and September 15th) in the 6 study locations in the Quoddy region between 2009 and 2015 ranged from 11 to 14.5°C (Fig. 6). Mean temperatures were the greatest in 2012 for all areas (Fig. 6). The warmest area was generally Passamaquoddy Bay, with the highest mean summer temperature (14.52 °C) was recorded in 2012 (Fig. 6).

A logistic regression revealed a significant ($Z_{31}=2.399$, $pseudo-R^2=0.24$, $P=0.016$) positive relationship between the presence of YOY cunner in bio-collectors from a particular study area and year and mean summer bottom temperature within that area and year (Fig. 7). The likelihood of finding YOY was predicted to be 50% at approximately 12.3°C (Fig. 7).

The density of YOY cunner in bio-collectors from the different study areas and years varied between 0 and 2.3 individuals m⁻². Highest densities were observed at

temperatures greater than 12°C, although low densities were also observed at temperatures $\geq 12^\circ\text{C}$ (Fig. 8). The highest density of YOY cunner occurred at a summer bottom temperature of 12.25°C, in Beaver Harbour in 2013 (Fig. 8). The density of YOY cunner increased exponentially with bottom temperature (Table 2; $R^2=0.15$, $P = 0.044$), and this function better described the relation between the two variables than did a linear regression (or the null intercept model), as determined by AIC (Table 2, $\omega_i=9.99 \times 10^{-1}$).

When the same analyses were run based on a 74.4 mm cut-off for YOY cunner, which is what was suggested by the BoF data in 2013, the same conclusions were reached as described above using a 66.0 mm cut off based on the NS cunner data. For example, there was a positive significant logistic regression ($Z_{31}=2.399$, *Pseudo- R^2* = 0.237, $P=0.019$) between the presence of YOY cunner and mean summer bottom temperature, and a 50% likelihood of cunner occurring at 12.3°C (Table 3). Also, an exponential model ($R^2=0.38$, $P = 0.0005$) was again the best of the three models tested to explain the relationship between density and mean summer bottom temperature (Table 3).

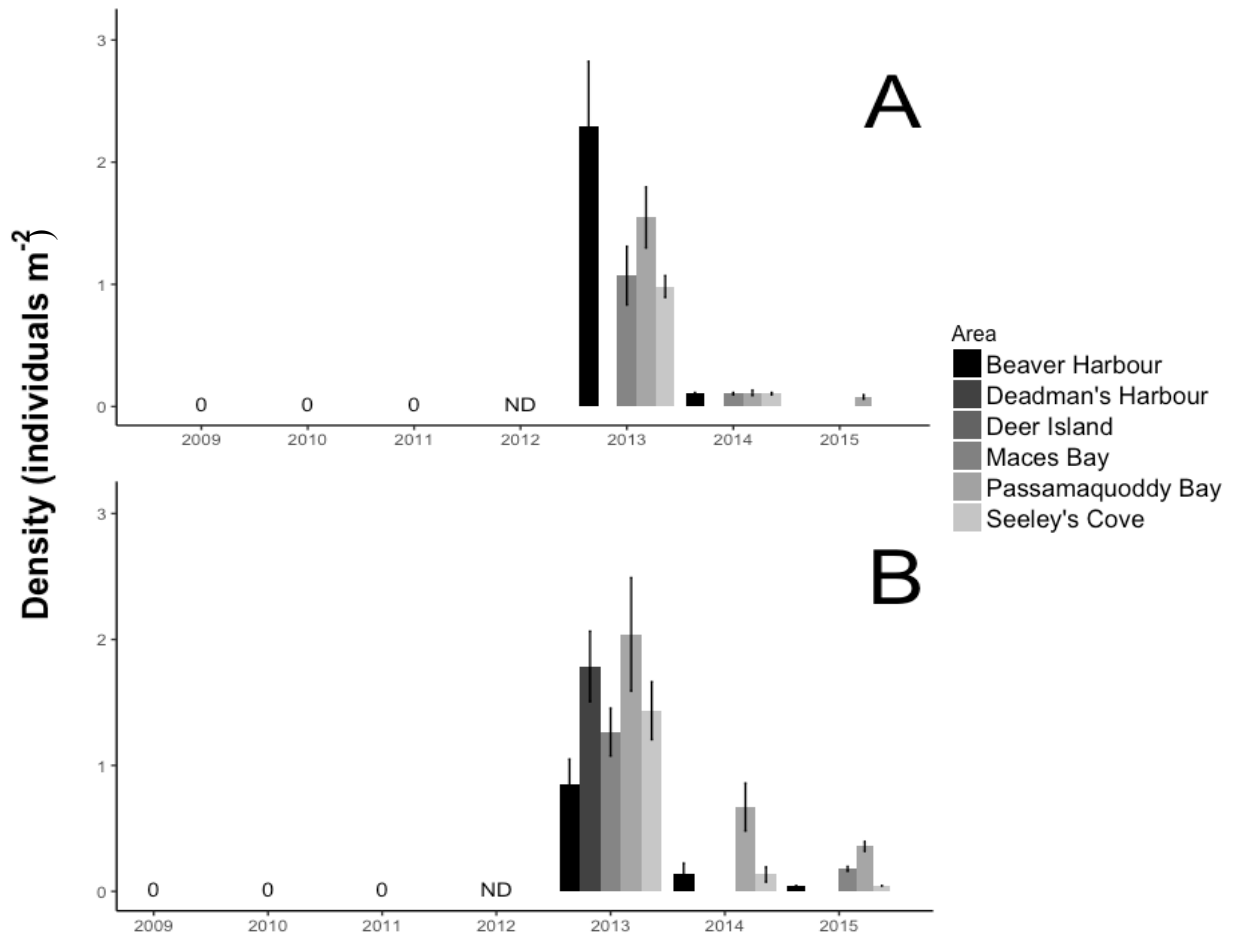


Figure 4: Mean ($\pm$ SE) density (individuals m^{-2}) of A) YOY and B) greater than one year of age cunner (*Tautoglabrus adspersus*) in bio-collectors deployed in 6 areas within the Quoddy region of the southwest Bay of Fundy from 2009 to 2015. Zeros indicate that there were no cunner collected, and ND indicate that cunner were captured but their density was not determined.

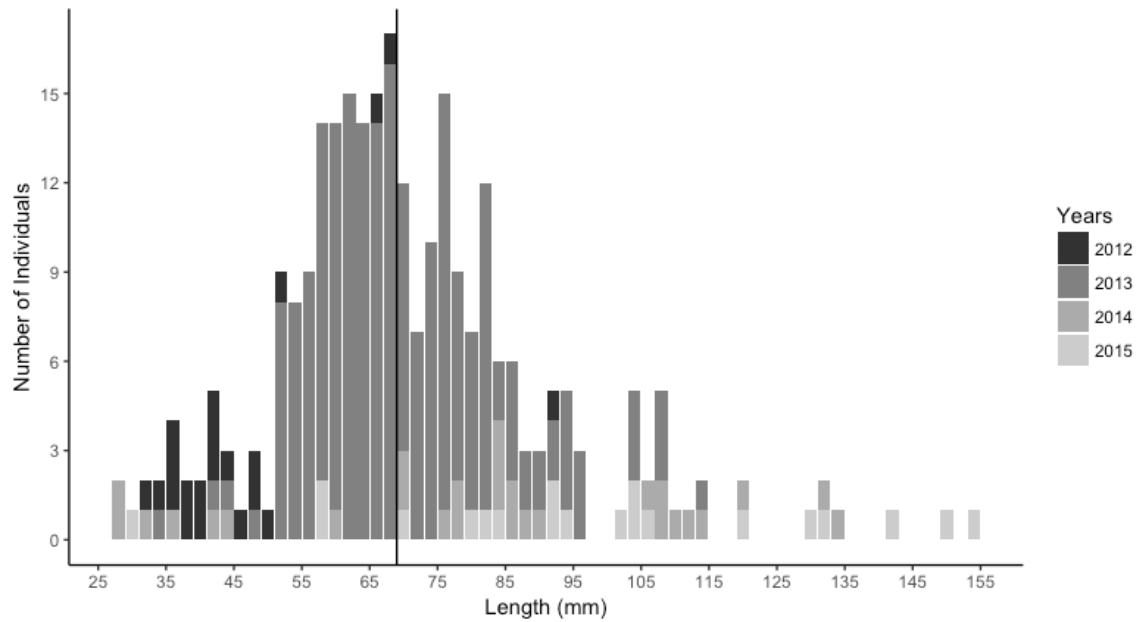


Figure 5: Length-frequency distribution of cunner (*Tautogolabrus adspersus*) caught in cobble-filled bio-collectors deployed in 6 areas within the Quoddy region of the Bay of Fundy from 2012-2015. All individuals to the left of the solid line are classified as YOY cunner based on NS cunner data (see Methods); individuals to the right of this line are considered to be in the 1+ age class.

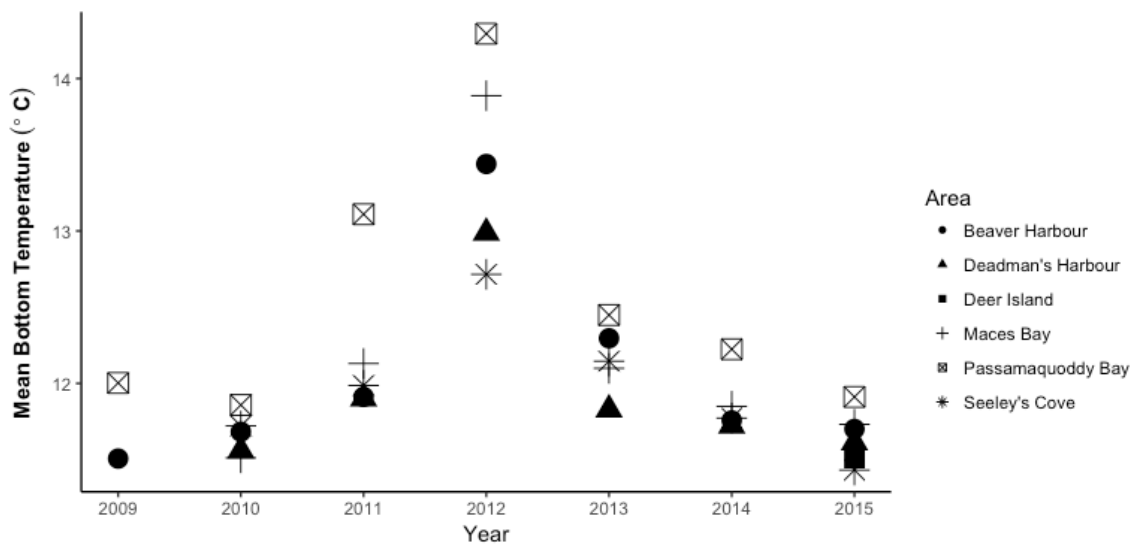


Figure 6: Mean daily summer bottom temperature (7 July to 15 September) from 2009 to 2015, at 20 sites within 6 study areas within the Quoddy Region where bio-collectors were deployed; there were 1-6 sites per study area. All temperature loggers were deployed within the same depth range (5 to 10 m).

Table 2: Comparison of linear, exponential and intercept only (null) model of the relationship between the density of YOY cunner (*Tautogolabrus adspersus*) in bio-collectors and the mean summer bottom temperature in six study areas and seven years (2009-2015) within the Quoddy region of the Bay of Fundy. AIC values adjusted for number of parameters (AIC_C), with lower values representing better fit between model and data, as well as AIC ω_i values, which show the relative support (weight) each model received.

Model	r^2	F	df	P	AIC_C	AIC weights (ω_i)
Linear regression	0.105	4.31	1, 26	0.092	47.3	1.62×10^{-07}
Exponential	0.137	4.48	1, 26	0.044	16.1	0.99
Intercept	-	-	-	-	49.6	5.14×10^{-08}

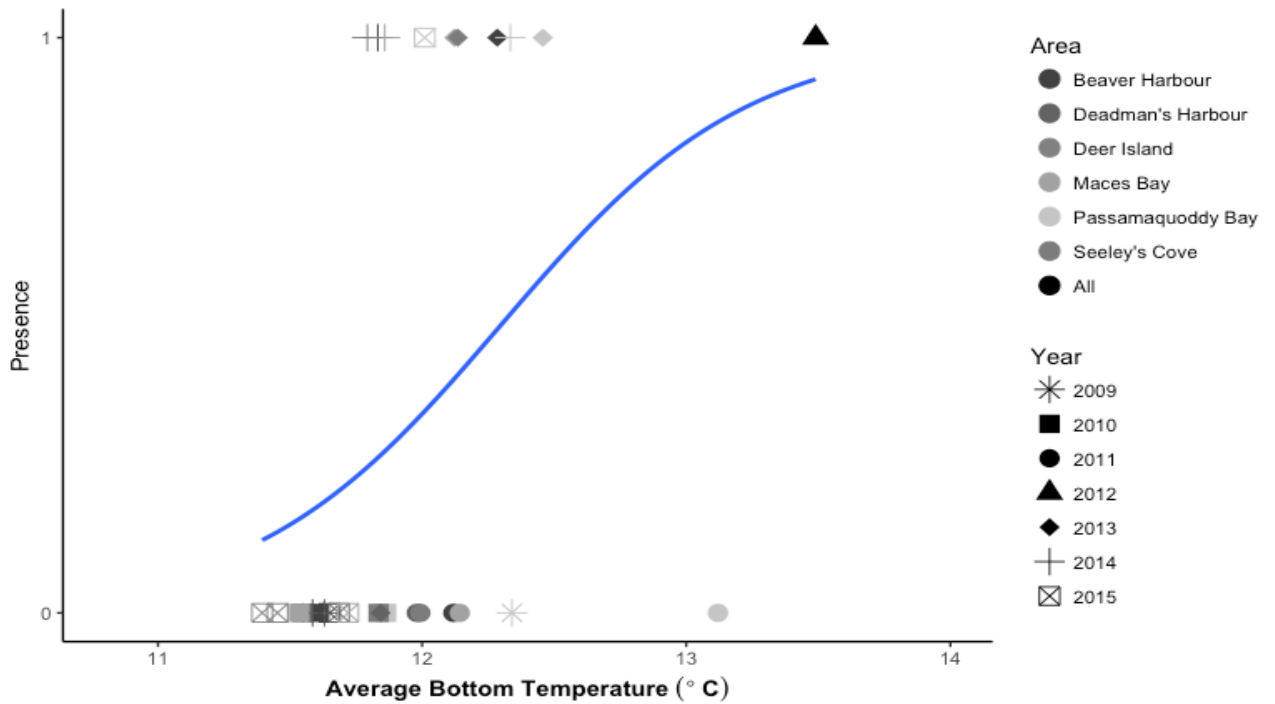


Figure 7: Presence (1) or absence (0) of YOY cunner (*Tautogolabrus adspersus*) in bio-collectors in relation to mean summer bottom temperature (°C) in 6 study areas within the Quoddy Region of the Bay of Fundy from 2009 to 2015. Given that cunner were not counted in 2012, a single point (black triangle) represents the presence of cunner that year plotted against the mean bottom temperature for all areas. The blue line represents a significant ($Z_{31}=2.399$, $pseudo-R^2=0.24$, $P=0.016$) logistic regression between the presence of YOY cunner and the mean bottom temperature.

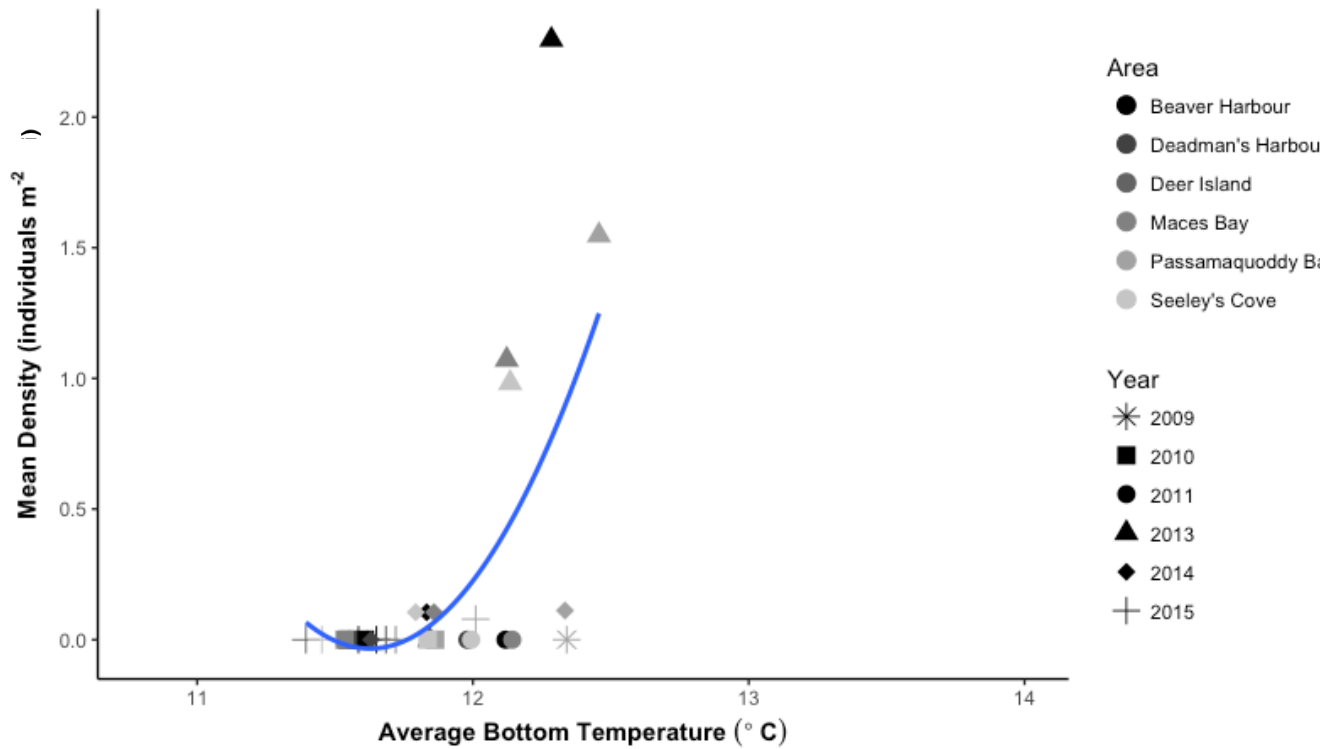


Figure 8: Mean density of YOY cunner (*Tautogolabrus adspersus*) in cobble-filled bio-collectors in relation to mean summer bottom temperature (°C) in 6 study areas within the Quoddy region of the southwest Bay of Fundy from 2009 to 2015 (2012 not included because individuals were not counted). The blue line represents a significant positive exponential ($R^2=0.15, P = 0.044$) relationship between the density of YOY cunner and average bottom temperature.

Table 3: Comparison of statistical results obtained using two “cut-off” lengths (66 and 74.4 mm) to determine the maximum size of YOY cunner (*Tautoglabrus adspersus*) in relating the presence/absence (logistic regression) and density (exponential function) of YOY cunner in bio-collectors to the mean summer bottom temperature in 6 study areas within the Quoddy Region of the Bay of Fundy from 2009 to 2015. The 66 mm and 74 mm length cut offs were obtained based on length frequency analysis of cunner caught in bio-collectors in the Scotian Shelf off Nova Scotia (St. Mary’s Bay, Lobster Bay and Cape Breton) in 3 areas from 2007-2014, and in 6 areas within the Quoddy Region of Bay of Fundy from 2009-2015, respectively.

Statistical test	66 mm	74 mm
Logistic regression	$Z_{31}=2.40$	$Z_{31}=2.14$
	$Pseudo- R^2=0.237$	$Pseudo- R^2= 0.130$
	$P=0.019$	$P=0.067$
Exponential	$F_{2,25}=4.480$	$F_{2,25}=7.704$
	$R^2=0.147$	$R^2=0.236$
	$P=0.0440$	$P=0.010$

Temperature-dependence of cunner embryo development in the laboratory

The first laboratory experiment was conducted to determine the timing of the beginning, peak and end of hatch of cunner embryos at temperatures ranging from 11 to 15°C to select the sampling times in the second experiment. In the first experiment, the first hatching occurred at 60 hpf (2.5 days post fertilization (dpf) at 15°C and at 72 hpf (3 dpf) at 11°C (Fig. 9). Consequently, 80 hpf (3.25 dpf) was used as the first sampling period in the second experiment, to estimate hatch success near the beginning of the hatch period at all experimental temperatures (Fig. 9). During the first experiment the highest proportion of hatch occurred at 120 hpf (5 dpf) regardless of temperature over the range (11-15°C) (Fig. 9), which was therefore selected as the second sampling point in the second experiment, as indicative of the peak of hatch. The final sampling point in the second experiment was determined to be 160 hpf (6.67 dpf), based on the large proportion of mortality that occurred by this time in all temperature treatments during the first experiment (Fig. 9). Mortality continued to occur after this time during the first experiment until the experiment was terminated after 228 hpf (9.5 dpf), by which time the majority of embryos had died. Between 160 and 228 hpf, a large proportion of embryos were classified as “missing” (Fig. 9), indicating these embryos had died and dissolved or broken as dead embryos were extremely fragile and prone to breakage.

In the second experiment, the proportion of hatched embryos increased with temperature in both rearing methods, i.e., the flow-through petri dish with 250 µm Nitex and still water in 150ml plastic beaker (Fig. 10). The proportion of hatched embryos at times of peak hatch (120 hpf) varied significantly among rearing treatments ($F_{4,103}=12.01$, $P=7.63 \times 10^{-4}$) (Fig 10). Because hatch was higher during time of peak hatch in

the still-water treatment than in the flow-through petri dishes, subsequent analyses of the effect of temperature and time on hatch were carried out solely using data from embryos reared in still water in beakers.

The proportion of embryos that hatched in beakers varied over time and with temperature, with the highest hatching success being 22% in one tank at 15°C at the sampling time of 120 hpf (Fig. 11). The proportion of embryos that hatched in these conditions increased over time, peaking and leveling off at 120 hpf, and with increasing temperature (11 to 15°C) (Fig. 11). The two-way nested ANOVA indicated that there was no significant difference in hatch rate between different tanks of the same temperature ($F_{1,10}=1.45$, $P=0.335$), but a significant positive effect of temperature ($F_{4,349}=16.03$, $P=1.59 \times 10^{-10}$) and time ($F_{2,351}=6.62$, $P=0.0019$). There was no significant interaction between time and temperature ($F_{8,345}=61.36$, $P=0.223$). Tukey HSD tests indicated that hatch success did not differ significantly between 11 and 12°C ($P=0.999$), or between temperatures of 13-15°C ($P_{13-14^\circ\text{C}}=0.987$, $P_{13-14^\circ\text{C}}=0.999$, $P_{14-15^\circ\text{C}}=0.996$), but was significantly higher at 13°C than at 12°C ($P=0.044$). Tukey HSD tests also indicated that the proportion of hatch was significantly ($P < 0.05$) lower at 80 hpf than at 120 and 160 hpf, but that hatch success did not differ significantly between 120 and 160 hpf ($P=0.697$). These results are similar to those from the first experiment where hatch peaked between 80 and 120 hpf and then remained more or less constant up to 160 hpf, indicating that the period of hatch was captured by the sampling periods chosen for the second experiment.

The relationship between the proportion of embryos that successfully hatched in the lab during the peak sampling times (120 and 160 hpf) and rearing temperature (11-

15°C) (Fig 10) was found to be better fit by an exponential ($\omega_i = 0.982$) than by a linear ($\omega_i = 0.018$) regression, as determined through AIC (Table 4).

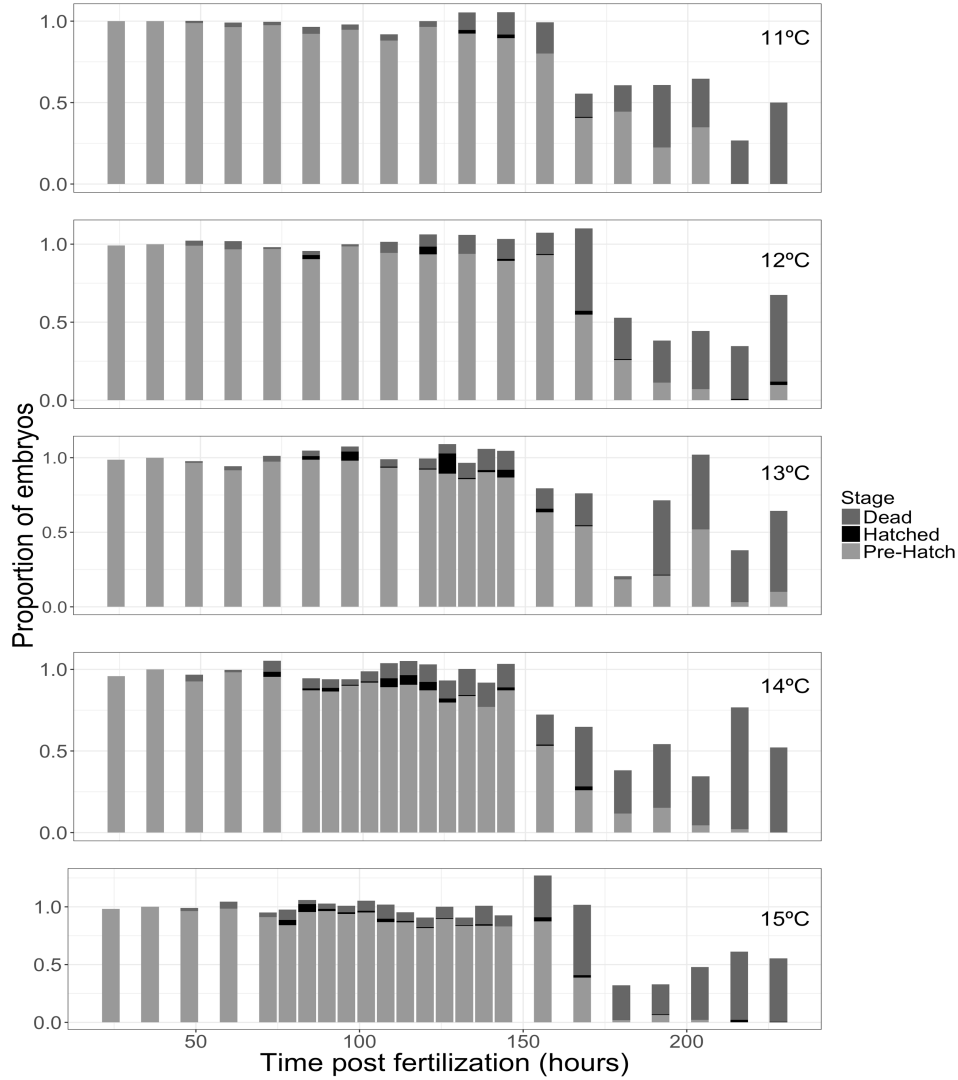


Figure 9: Results of first laboratory experiment, which were used to estimate the hatch period of cunner (*Tautoglabrus adspersus*) embryos at 11°C to 15°C. Mean (among 3 petri dishes from 3 tanks of the same temperature) proportion of pre-hatch, hatched, and dead cunner embryos over a period of 228 hours post fertilization (hpf) at each of 5 temperature treatments (11, 12, 13, 14, and 15°C). Sampling was done every 12 hours until the first eleutheroembryo hatched, and then every 6 hours until 2 successive samplings revealed no new hatches, at which point sampling frequency returned to every 12 hours, until the end of the experiment

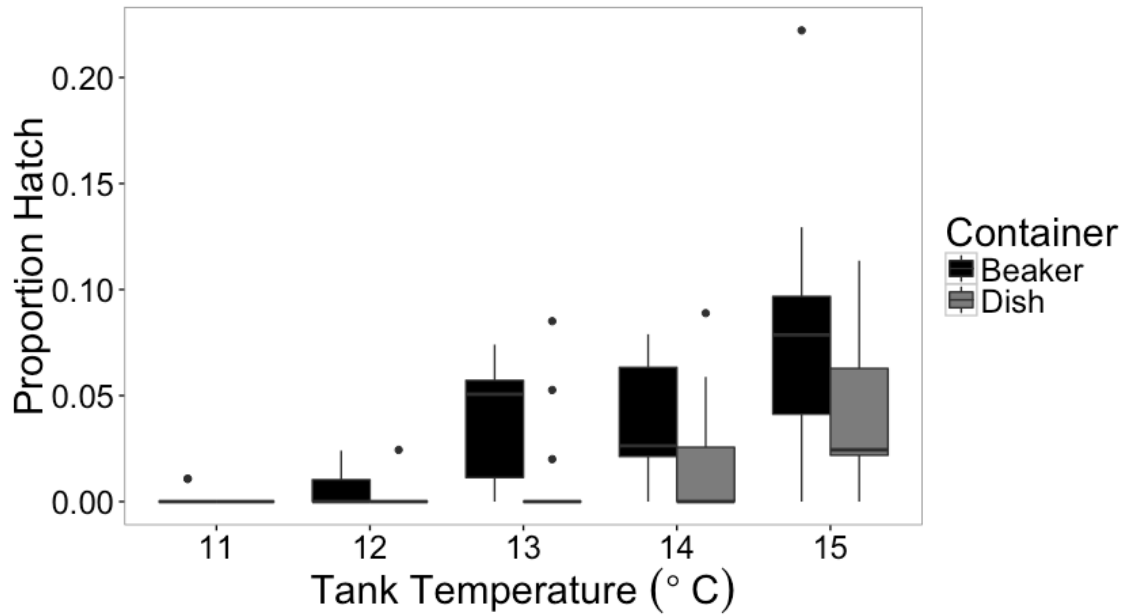


Figure 10: Boxplots showing the proportion of cunner (*Tautogolabrus adspersus*) embryos that hatched at 120 hpf (time of peak hatch) in two rearing treatments (beakers and dishes) at temperatures of 11-15°C (n=3 tanks per temperature, n=1 dish per tank) in the second laboratory experiment (n=3 beakers and n=5 petri dishes per tank). Each horizontal line within a box represents the median proportion of embryos that hatched, boundaries of the box indicate the 25th -75th percentile (inter-quartile range) and the whiskers indicate the highest and lowest quartiles. Black circles seen above the boxes are outliers that are outside the whiskers.

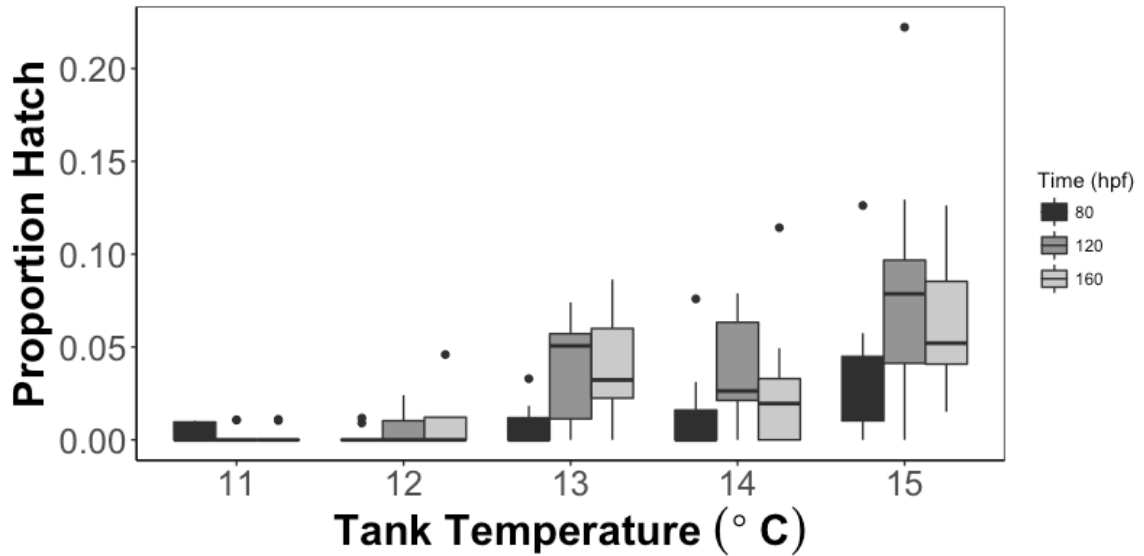


Figure 11: Boxplots showing the proportion of cunner embryos (*Tautoglabrus adspersus*) that hatched when reared in beakers in still water at temperatures ranging from 11 to 15°C (three tanks per temperature) in the second laboratory experiment. Sampling was carried out at 80 (black), 120 (gray) and 160 (light gray) hours post fertilization (hpf). Embryos were reared in nine 150 mL plastic beakers per tank at each temperature, and three beakers per tank were sampled (destructively) at each sampling time (80, 120 and 160 hpf) to calculate the mean proportion hatch. Each horizontal line within a box represents the median proportion of embryos that hatched, boundaries of the box indicate the 25th -75th percentile (inter-quartile range) and the whiskers indicate the highest and lowest quartiles.

Comparison of the field observations and the laboratory experiment

The exponential function developed based on the laboratory data ($\text{Hatch} = e^{0.129(\text{Temperature}) - 3.739}$) (Fig. 11) was used to estimate the potential hatch of cunner embryos in the field based on the mean summer bottom temperatures observed for each combination of study area ($n = 6$) and year ($n = 7$) in the Quoddy region of the Bay of Fundy. This estimated potential hatch was then related to observed YOY cunner density in the bio-collectors, and a significant exponential relation ($R^2=0.34$, $F_{1,25}=12.94$, $P=0.014$) was found between these two variables across the different combinations of years and study areas (Fig. 12); AIC showed that this exponential function ($\omega_i = 9.99 \times 10^{-1}$) better described the data than a linear function ($\omega_i = 2.00 \times 10^{-7}$) (Table 5).

Table 4: Comparison of the linear, exponential and intercept only (null) models predicting hatching success of cunner embryos 120 hours post fertilization at rearing temperatures of 11, 12, 13, 14, 15°C during the second laboratory experiment. AIC values adjusted for number of parameters (AIC_c), with lower values representing better fit between model and data, as well as AIC ω_i values, which show the relative support (weight) each model received.

Model	r²	F	df	P	AIC_c	AIC weight (ω_i)
Linear	0.369	51.36	1, 88	2.25x10 ⁻¹⁰	-170	1.80 x10 ⁻⁰²
Exponential	0.442	34.04	2, 87	6.37x10 ⁻⁷	-178	0.98
Intercept	-	-	-	-	-150	8.65x10 ⁻⁰⁷

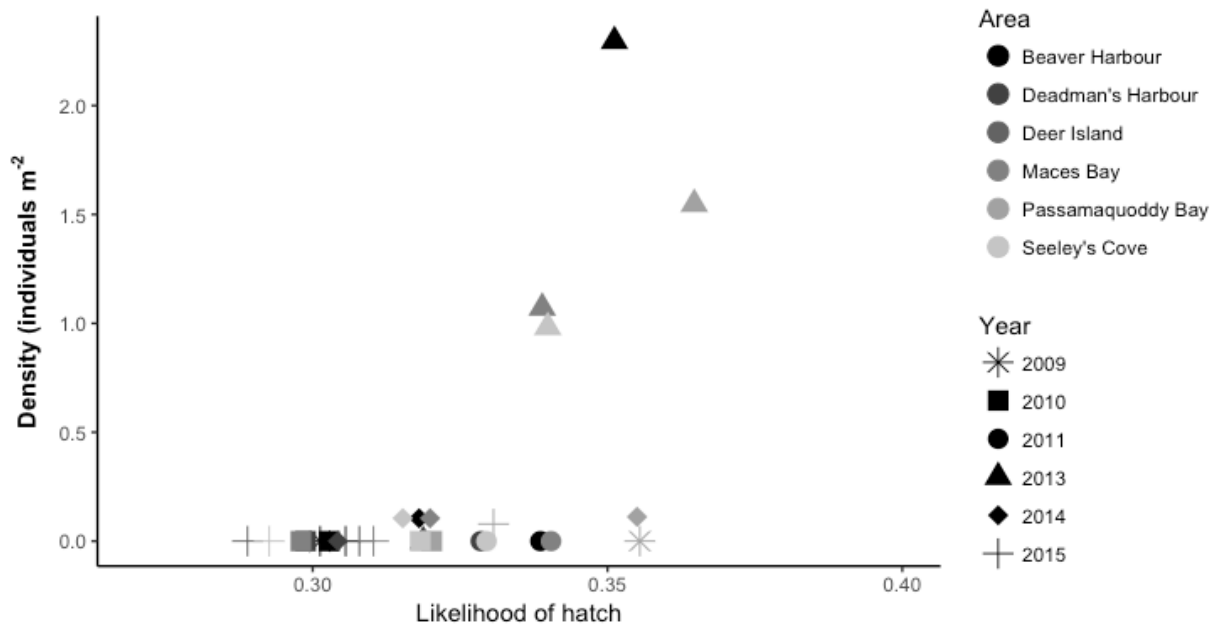


Figure 12: Relationship between the likelihood of hatch of cunner (*Tautoglabrus adspersus*) embryos, at temperatures recorded at the same areas and years, and the density of YOY cunner (individuals m⁻²) observed in bio-collectors in different years and areas of the Bay of Fundy Quoddy Region. The likelihood of hatch was estimated based on results of a lab experiment estimating hatch success at different temperatures and mean summer temperature recorded in the different study areas and years.

Table 5: Comparison of the linear, exponential, and intercept only (null) models predicting the density (individuals m⁻²) of YOY cunner in the bio-collectors based on the exponential relation between temperature and hatch success derived during the second laboratory experiment ($\text{Hatch} = e^{0.129 (\text{Temperature}) - 3.739}$) and the mean summer bottom temperatures (July 7th to September 15th) observed in nature for each combination of area and year. AIC values adjusted for number of parameters (AIC_c), with lower values representing better fit between model and data, as well as AIC ω_i values, which show the relative support (weight) each model received.

Model	r²	F	df	P	AIC_c	AICc weights (ω_i)
Linear	0.15	18.6	1, 104	3.75x10 ⁻⁰⁵	40.4	2.00x10 ⁻⁰⁷
Exponential	0.34	913	2, 103	1.38x10 ⁻⁰³	9.62	0.99
Intercept	-	-	-	-	48.8	3.08 x10 ⁻⁰⁹

Discussion

In the present study, field observations and laboratory experiments provided evidence that cunner, *Tautoglabrus adspersus*, is undergoing a range expansion into the Bay of Fundy, and that this range expansion is consistent with the hypothesis that increasing water temperature enabled the successful development of their pelagic embryos. Using data from a bio-monitoring program of cobble-bottom habitat in the Quoddy region of the BoF, initiated in 2009, I found the first record of presence of juvenile cunner in 2012, when July-September bottom temperature varied from 11.7 and 14.2°C. My laboratory experiment demonstrated that hatching success of cunner embryos varies with temperature from 11 to 15°C, and increases markedly at approximately 13°C. I found a significant positive relationship between the occurrence and density of juvenile cunner detected by the monitoring program and summer bottom temperature in different sampling areas and years in the Quoddy region. The probability of cunner embryo hatching was estimated based on bottom temperature and the temperature-hatch relationship determined in the laboratory. YOY densities observed in the field were positively linked to the calculated likelihood of hatch from the laboratory experiment, providing evidence that temperature-dependent embryo development is the mechanism for the range expansion of cunner into the BoF.

Occurrence of cunner in the Quoddy region of the Bay of Fundy

The literature points to the occurrence of cunner, especially juveniles, within the Bay of Fundy being rare, with limited information indicating the presence of adults

having been collected from the 1920s until 2001 (Collette & Klein-MacPhee 2002). Studies that have monitored biodiversity of demersal fishes in the Quoddy Region of the BoF, with sampling tools including bottom trawl, beach seine and dive transects, have reported no YOY cunner in this region from 1970 to 2012 (MacDonald *et al.* 1984, Arens 2007, Jennings & Hunt 2010, Ricard & Shackell 2013, Ipsen 2013), while studies using the same sampling gear to sample fish assemblages along the Nova Scotia coast of the BoF did catch cunner (Horne and Campana 1989). It should be noted that cunner embryos have been sampled in Saint John Harbour, approximately 40 km further into the BoF, suggesting that this early life stage may have been present in the Quoddy region over this period (Whitford, 2008, Van Guelpen unpubl.). However, early stages of cunner embryos can be difficult to distinguish from yellow flounder (*Pleuronectes ferruginea*), which also occurs in the area (Fahay 1983). If cunner embryos were identified correctly and were present on the New Brunswick side of the BoF prior to 2012, the lack of YOY cunner sampled in bio-collectors (including Saint John Harbour in 2010, Hunt and Rochette unpubl. data) suggests these embryos may have been unable to survive.

It was therefore a surprise when a bio-monitoring program of cobble-bottom habitat in the Quoddy regions of the Bay of Fundy sampled a large number of YOY cunner in 2012 (approximately 30 mm in size); counting these juvenile cunner was not part of the protocol at the time, but four technicians and researchers that were involved in processing the 2012 bio-collectors all independently recall cunner being collected in the 10s to 100s of individuals. This program had sampled 0 YOY cunner in a total of 687 bio-collectors that were deployed in the same study areas in 2009, 2010 and 2011,

providing strong evidence that YOY cunner first appeared in the study area in 2012, and they did so in high numbers.

YOY cunner found in the bio-collectors were almost certainly spawned near where they were sampled. Due to the fast rate of embryo development (72 hours at 15°C) and the residual current patterns within the BoF that carry larvae in a counterclockwise circulation (Xue *et al.* 2008), cunner embryos spawned in Saint Mary's Bay NS, the nearest reproductive population (Horne and Campana 1989), would be unlikely to disperse to the Quoddy region of the BoF. The absence of YOY cunner in our samples prior to 2012 does not preclude the possibility that older cunner were present in our study area in those earlier years, as individuals larger than ≈ 160 mm in total length may have been too large to enter our passive bio-collectors. The possible presence of such large cunner in the absence of YOY individuals would likely be the result of migration into the Quoddy region of the Southern BoF from elsewhere. Presence of occasional adults would not constitute range expansion because there were no signs of a reproducing population in the lower BoF (Sorte *et al.* 2010). Therefore, YOY cunner in the bio-collectors provide evidence of recent establishment of a reproducing population and range expansion into the Quoddy region of the Southern BoF.

Given that sampling was consistent over time, I interpret the first occurrence of YOY cunner in the bio-collectors in 2012 as evidence that the species' range has recently expanded into the Quoddy region of the Southern BoF rather than as a result of a change in sampling design. In the field component of this study, the nature of the sampling tool (bio-collectors), sampling locations, as well as sampling period and

intensity did not markedly differ between years. Areas investigated were sampled annually from either 2009 (Passamaquoddy Bay and Beaver Harbour) or 2010 (Seeley's Cove, Maces Bay and Deadman's Harbour) to 2015. Therefore, the change in cunner presence/absence and density in the bio-collectors cannot be attributed to a change in sampling locations, as study areas and sites were largely the same over time. While the specific deployment and retrieval days varied somewhat from year to year, the general sampling period (deployment late June to early July; retrieval mid-October to mid-November) was the same across years, both before (2009-2011) and after (2012-2015) the appearance of cunner in the bio-collectors. The period of deployment of the collectors (July-October) closely spans the cunner spawning season (July-September) and the timing of settlement of juvenile cunner to the bottom (August-October) (Johansen 1925, Collette & Klein-MacPhee 2002). The intensity of deployment of bio-collectors was the only aspect of the sampling protocol that changed to some degree before and after the appearance of cunner. In 2009-2011, prior to cunner appearance, the yearly mean number of bio-collectors deployed at the study locations was 229. After 2011, mean number of bio-collectors deployed at these locations increased to 268. However, the smaller number of bio-collectors prior to the observation of cunner was entirely due to a much lower sampling intensity during the first year of this study (2009), when only 152 bio-collectors were deployed; if we exclude this year, the mean number of bio-collectors deployed in the two years (2010 and 2011) prior to the observation of cunner (264 bio-collectors) is nearly the same as that in the three years (2012, 2013, 2014) when cunner were observed (268 bio-collectors) (Table 1).

Similar sampling on the Scotian Shelf in Nova Scotia provides additional circumstantial evidence that the absence of cunner in our samples from the Quoddy region in 2009, 2010 and 2011 was not related to sampling methodology, but rather reflected an absence of individuals in this region, as YOY cunner were caught in all years (2009-2015) that bio-collectors were deployed on the Scotian Shelf, and in some cases as many as 42 individuals m^{-2} in a single bio-collector (Hunt *et al.* 2017, unpublished data, J. Tremblay DFO). Interestingly, the density of juvenile cunner in bio-collectors deployed on the Scotian Shelf was lower between 2009-2011 (7.21 individuals m^{-2}), the years when cunner were absent in the BoF samples, than between 2012-2015 (15.23 individuals m^{-2}), the years when cunner were present in the BoF samples (unpublished data, J. Tremblay DFO). Increases in the density of juvenile cunner in Nova Scotia bio-collectors may also result from the increase in sea-surface temperature seen throughout Atlantic Canada, including the Scotian Shelf, in 2012 (Mills *et al.* 2013).

Potential importation of YOY cunner

The timing of the appearance of YOY cunner in bio-collectors in the Quoddy region of the BoF is not consistent with anthropogenic introduction of cunner by studies assessing the potential for use of cunner as a cleaner fish in Atlantic salmon aquaculture. A hatchery was established in Saint Andrews, NB, within the Quoddy region of the Southern BoF in 2011, but did not produce embryos and juveniles until 2013. In addition, all outflows from the hatchery lead to fresh water; therefore, any embryos that escaped would almost certainly have died. The facility had adults within the hatchery

starting in 2011 (imported from St. Mary's Bays N.S.), which were held in a recirculating system, preventing any escapes of adults into the Quoddy region of the Southern BoF. Therefore, this hatchery could not have led to the appearance of YOY cunner in bio-collectors beginning in 2012. The goal of the hatchery is to use cunner in sea pens for sea lice removal from Atlantic salmon being held in commercial aquaculture pens in the lower BoF. No cunner were introduced to aquaculture pens in the Quoddy region of the BoF during the summers of 2011-2015; only lab studies were conducted from 2013 to 2015 (Kelly Cove LTD. pers. comm., 2017). The only field trials done with adult cunner in salmon pens in the Quoddy region of the BoF occurred from summer 2009 to spring 2010. Although there is a small chance that adult cunner escaped from the pens or spawned while being held in the field, no YOY cunner were detected in bio-collectors in 2009, 2010, or 2011. Therefore, the presence of YOY cunner in bio-collectors in the Quoddy region of the Southern BoF is not consistent with anthropogenic introduction, but rather with range expansion.

Occurrence and density of YOY cunner and relationship to temperature

Because of difficulty obtaining accurate coastal SST through satellite imagery, bottom temperature, recorded during the deployment of the bio-collectors, was used instead. Inter-annual variability in bottom and sea surface temperature are strongly correlated in the Gulf of Maine, including the BoF, especially on the continental shelf and upper slope (LeBris *et al.* 2017; Richaud *et al.* 2016). In our study area, in particular, sea surface temperature and shallow bottom temperature as assessed in this

study (less than 10-15 m relative to chart datum) are not only strongly correlated over time, but they are actually very similar, given the limited stratification due to strong tidal mixing in the BoF, which results in a thermally uniform water column (Wahle *et al.* 2013).

It has been proposed in earlier work that the absence of cunner from the Quoddy region of the Southern BoF was caused by the water temperature being too cold to support the development of embryos (Collette & Klein-MacPhee 2002). Under this hypothesis, the occurrence and density of YOY cunner in bio-collector samples from 2012 to 2015 suggest an increase in temperature in recent years that allowed cunner embryos to successfully develop within the surface waters of the Quoddy region of the Southern BoF. My results are consistent with this expectation, as I found a significant positive relationship between both the occurrence (presence/absence) and the density of YOY cunner, and summer bottom temperature in different years and areas. These findings strongly suggest that temperature plays an important role in determining whether YOY cunner will recruit to an area in the Quoddy region of the Southern BoF, with warmer water increasing the probability that embryos survive and successfully develop into larvae. Interestingly, it had been hypothesized that cunner embryos require temperatures of 13°C or more to develop successfully (J. Green, pers. com. in Collette & Klein-MacPhee 2002), and although my study does not support the existence of a hard threshold for cunner embryo development, both its lab and field components indicated marked increased in hatching success at this proposed threshold of 13°C.

Embryonic development in a laboratory setting

To test the hypothesis that the range of cunner is expanding into the BoF because increasing water temperature is enhancing embryo development, I reared cunner embryos at a range of temperatures in two types of containers, still-water beakers and flow-through dishes, in the laboratory. The beakers were selected based on a previous study that investigated cunner embryo development in still water (Gutjahr-Gobell *et al.* 2002), while the dishes were based on the use of Nitex to house embryos in a flow-through system in the hatchery from which the cunner eggs were obtained. Hatching success was significantly greater in beakers compared to dishes during peak time of hatch (120 and 160 hpf). The lower hatching success in the dishes was potentially caused by the surface tension from the small size of the Nitex (250 μm) covering the sides of the dishes, which may have prevented water flow and oxygen supply to the embryos (Gutjahr-Gobell *et al.* 2002). In contrast, the buoyant embryos remained at the surface of the water in the beaker, and were thus likely well oxygenated. Because of the greater mortality in the dish rearing treatment, data from the dishes was used only to determine the timing of hatch for the design of the second laboratory experiment.

The hatching success of cunner embryos in my second laboratory experiment (average of 5.9 % at 15°C) compares favourably with that of previous studies examining development of cunner embryos, which provides confidence that the embryos used in my study were healthy and the conditions under which they were held were suitable for hatching. An earlier study (Williams and Williams, 1973) of cunner embryo development reported a mean hatch success of 5% at 15°C, and the hatchery from which

my embryos were obtained has an average 8% hatching success at this temperature (Kelly Cove LTD. pers. comm., 2015). Demersal fish, such as cunner, generally produce a very high quantity of embryos that are low in reserves (small size at hatch and small yolk sac), in comparison to benthic fish that often brood or protect their embryos on the bottom and produce a smaller number of eggs with greater reserves (Murua and Saborido-Rey, 2003). The reproductive strategy of many eggs with low survival is considered successful if at least one of the offspring makes it to adulthood (Holden and Raitt, 1974, Collette & Klein-MacPhee 2002).

In my second lab experiment, a greater proportion of embryos developed into eleutheroembryos at higher water temperatures. The temperatures used in this lab study (11 to 15°C) were chosen to reflect the range of mean summer bottom temperatures observed in the Quoddy region of the Southern BoF during the field component of the study. A very small proportion (0.2%) of embryos successfully hatched at the colder temperatures of 11 and 12°C, while markedly more embryos hatched in warmer treatments of 13°C (2.7%), 14°C (2.7%), and 15°C (5.8%). It is worth noting that hatch success at 11 and 12° C was not 0, indicating that the 13°C thermal threshold proposed for successful cunner embryo development (Gutjahr-Gobell *et al.* 2002; Collette & Klein-MacPhee 2002) is not a hard threshold, although success is clearly very low at such low temperature and increased markedly beyond it. This is especially true in Newfoundland, where there is a large population of cunner in Conception Bay (Collette & Klein-MacPhee 2002) While in the most part summer water temperatures can be above 13° C (Mason *et al.* 1999), average temperature can decrease to below this ideal

temperature, yet there is a reproductive population in this area. Due to Newfoundland being the northern limit of the species range, cunner embryo development in this region could have adapted to the colder water temperature. My study did not examine the upper temperature limits for successful hatch of cunner embryos, but previous research suggests that embryo rearing is not successful at temperatures above 22°C (Williams and Williams, 1973).

Comparison of field observations and laboratory experiment

While most studies of range expansions explore causality by performing correlations between the occurrence or density of a species and temperature (Rayner *et al.* 2003; Nye *et al.* 2009; Richards *et al.* 2012; Pershing *et al.* 2015), I have taken one step further to try to understand the mechanism behind the range expansion of cunner. This project not only documented a relationship between increasing temperature in the Quoddy region of the BoF and increased occurrence and density of YOY cunner in a field monitoring program (bio-collectors), but also tested (and verified) a hypothesized mechanism for this relationship. Specifically, my study showed a significant positive relationship between the hatch success of cunner embryos at different temperatures in the lab and the occurrence and density of YOY cunner at the same mean summer temperatures in nature. This positive relationship supports the hypothesis that the absence (up until recently) of YOY cunner in the Quoddy region of the Bay of Fundy was at least partly due to the inability of embryos to successfully develop, hatch and survive in the region's cold waters. By demonstrating a significant link between the field

and lab portions of this project, I can support the hypothesis that the range expansion of cunner into the Bay of Fundy is at least partly due to increasing water temperature enabling successful embryo development and hatch.

Ecological effects of the range expansion of cunner

The impacts of cunner on the ecosystem were not investigated in this study, nor was it determined if the presence of cunner reduced or altered biodiversity, as is sometimes observed when non-native species colonize an area (Cohen and Carlton, 1997; Sorte *et al.* 2010). Forbes and Hunt (unpublished) investigated the assemblage of decapod crustaceans and fish colonizing bio-collectors in Beaver Harbour in the Quoddy region of the Bay of Fundy in both 2009 (previous to cunner arrival) and 2014 (after the expansion of cunner), and found that decapod and fish assemblages remained unchanged over this time period.

Conclusion

The present study provides evidence that the range of the cunner *Tautoglabrus adspersus* has recently expanded into the Quoddy region of the Southern BoF, and that the mechanism underlying this range expansion may relate to an increase in seawater temperature that is increasing successful development and hatch of embryos. This study differs from most previous studies on range expansions because it identified, through a combination of lab experiments and field observations, a likely mechanism for how the species is now able to inhabit the new area in which it was recently found. In contrast,

most studies on range expansion only correlate warming temperatures to the change observed in the species range over time, which does not provide a mechanistic explanation for the range change.

Warming ocean temperatures are expected to result in an increasing number of species shifting their range into new locations. A meta-analysis investigating species ranges from 1926 to 2008 found 36 coastal fish species undergoing range expansion, similar to cunner, and quantified that 75% of these shifts were pole-ward, consistent with effects of increasing water temperature in relation to climate change (Sorte *et al.* 2010). Similarly, Nye *et al.* (2009) were able to correlate the mean change in range of 24 out of 36 commercially valued fish stocks within the Northern Atlantic (including the Gulf of Maine) to large scale increases in temperature. Prior to my study, no study had documented the shift of a fish species into the Quoddy region of the Southern BoF, even though it is adjacent to one of the fastest warming portions of the world's oceans, the Gulf of Maine (Hobday and Pecl, 2014). It is possible that increasing temperature has enabled range expansion of cunner into the Quoddy region of the Southern BoF and potentially more species. More studies should be conducted to assess changes in benthic fish communities in the BoF, and to determine the mechanisms underlying changes in species' ranges.

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Curriculum Vitae

Candidate's full name: Krystal D. Woodard

Universities attended:

University of New Brunswick, Saint John: BSc (Hons), Marine Biology 2010-2014

Publications:

Clements, J. C., **Woodard, K. D.**, & Hunt, H. L. (2016). Porewater acidification alters the burrowing behavior and post-settlement dispersal of juvenile soft-shell clams (*Mya arenaria*). *Journal of Experimental Marine Biology and Ecology*, 477, 103-111.

Conference Presentations:

Woodard KD, Hunt, HL and Rochette, R. Atlantic Canadian Coastal and Estuarine Science Society, St. Andrews, NB. May 10-12, 2015. Investigating the role of temperature-mediated embryo development on the range expansion of cunner into the Bay of Fundy. Awarded Best Graduate Student Poster

Woodard KD, Hunt, HL and Rochette, R. Atlantic Canadian Coastal and Estuarine Science Society, Charlottetown PEI May 5-7, 2016. Investigating the role of temperature-mediated embryo development on the range expansion of cunner into the Bay of Fundy. Presentation

Woodard KD, Hunt, HL and Rochette, R. International Temperate Reefs Symposium, Pisa Italy, June 25-29, 2017. Investigating the role of temperature-mediated embryo development on the range expansion of cunner into the Bay of Fundy. Presentation