Effects of elevated CO₂ on early life history development of the yellowtail kingfish, *Seriola lalandi*, a large pelagic fish

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An increasing number of studies have examined the effects of elevated carbon dioxide (CO₂) and ocean acidification on marine fish, yet little is known about the effects on large pelagic fish. We tested the effects of elevated CO₂ on the early life history development and behaviour of yellowtail kingfish, *Seriola lalandi*. Eggs and larvae were reared in current day control (450 µatm) and two elevated CO₂ treatments for a total of 6 d, from 12 h post-fertilization until 3 d post-hatching (dph). Elevated CO₂ treatments matched projections for the open ocean by the year 2100 under RCP 8.5 (880 µatm CO₂) and a higher level (1700 µatm CO₂) relevant to upwelling zones where pelagic fish often spawn. There was no effect of elevated CO₂ on survival to hatching or 3 dph. Oil globule diameter decreased with an increasing CO₂ level, indicating potential effects of elevated CO₂ on energy utilization of newly hatched larvae, but other morphometric traits did not differ among treatments. Contrary to expectations, there were no effects of elevated CO₂ on larval behaviour. Activity level, startle response, and phototaxis did not differ among treatments. Our results contrast with findings for reef fish, where a wide range of sensory and behavioural effects have been reported. We hypothesize that the absence of behavioural effects in 3 dph yellowtail kingfish is due to the early developmental state of newly hatched pelagic fish. Behavioural effects of high CO₂ may not occur until larvae commence branchial acid–base regulation when the gills develop; however, further studies are required to test this hypothesis. Our results suggest that the early stages of kingfish development are tolerant to rising CO₂ levels in the ocean.

Keywords: behaviour, carbon dioxide, larval development, morphology, ocean acidification.

Introduction

Ocean acidification, caused by the uptake of anthropogenic carbon dioxide (CO₂) from the atmosphere, will affect the performance of many marine organisms, with implications for population dynamics, community structure, and ecosystem function (Doney et al., 2009; Gaylord et al., 2015). Atmospheric CO₂ concentrations were <300 ppm at the start of the 20th century, but have been rising steadily since then due to continued anthropogenic CO₂ emissions, reaching 400 ppm in 2014 (www.esrl.noaa.gov/gmd/ccgg/trends/) for the first time in at least 800 000 years (Luthi et al., 2008). CO₂ levels in the surface ocean are rising at the same rate as the atmosphere (Doney et al., 2009) and the continued uptake of additional CO₂ has caused ocean pH to decline by 0.1 units (Rhein et al., 2013). If the current rate of anthropogenic CO₂ emissions is maintained, climate models project that CO₂ concentrations in the atmosphere and surface ocean will exceed 900 ppm by the end of this century and could reach 1900 ppm by 2250 (Meinshausen et al., 2011).
Fish are generally considered to be relatively tolerant to rising CO₂ levels and declining seawater pH because of their well-developed mechanisms for acid–base regulation. Juvenile and adult fish actively regulate the concentrations of acid–base relevant ions in the blood and tissues to prevent acidosis in a high CO₂ environment (Heuer and Grassel, 2013) and this may help them cope with projected future CO₂ levels in the ocean (Menzel et al., 2009). Early life stages, however, may be more susceptible to high CO₂ because they are still developing their physiological regulatory processes (Brauner, 2008). Consequently, much of the recent research into the effects of ocean acidification on fish has concentrated on embryos, larvae, and small juveniles. While some studies have found no effects, or only minor effects, of high CO₂ on growth, development, and survival of fish early life history stages (Munday et al., 2009a, 2011; Franke and Clemmesen, 2011; Frommel et al., 2013; Bignami et al., 2013, 2014; Hurst et al., 2013; Maneja et al., 2013; Pope et al., 2014), other studies have reported negative effects on embryonic development (Forsgren et al., 2013), yolk provisioning (Chambers et al., 2014), tissue and organ development (Frommel et al., 2012; Chambers et al., 2014), swimming duration (Pimentel et al., 2014), and growth and survival (Baumann et al., 2012). However, most of these negative effects have been observed at relatively high CO₂ levels (≥1500 μatm CO₂), and sometimes only at very extreme levels (>4000 μatm CO₂). Nevertheless, these studies indicate that early life history stages of some species may be more susceptible to ocean acidification than others, especially in locations that may be exposed to periods of enhanced CO₂ from upwelling or eutrophication.

An increasing number of studies have also observed effects of elevated CO₂ on fish sensory systems and behaviours (Munday et al., 2014). When exposed to high CO₂ for more than a few days, there are changes in olfactory (Munday et al., 2009b), auditory (Simpson et al., 2011), and visual function (Chung et al., 2014). Activity levels (Munday et al., 2010), phototaxis (Forsgren et al., 2013), response to chemical cues (Dixson et al., 2010; Ferrari et al., 2011a), and startle behaviour (Allan et al., 2013) are all affected in larval fish. These changes in behaviour can affect the outcome of predator–prey interactions (Ferrari et al., 2011b; Allan et al., 2013), leading to significantly increased rates of mortality (Munday et al., 2010). Most research on behavioural effects of high CO₂ have been conducted on coral reef fish, but sticklebacks (Jutfelt et al., 2013), sharks (Dixson et al., 2014; Green and Jutfelt, 2014), and rockfish (Hamilton et al., 2014) also exhibit altered behaviours when continuously exposed to elevated CO₂ for short periods of time. Importantly, many of the effects on fish sensory abilities and behaviour occur below 1000 μatm, well within the range of CO₂ levels projected to occur by 2100 as a result of anthropogenic CO₂ emissions. Therefore, behavioural effects of rising CO₂ levels may be a more immediate threat to marine fish than effects on life history traits.

The effects of high CO₂ on early life history stages of commercially important species have been examined in cod (Frommel et al., 2012, 2013; Maneja et al., 2013), herring (Franke and Clemmesen, 2011), wallleye pollock (Hurst et al., 2012), European sea bass (Pope et al., 2014), and summer flounder (Chambers et al., 2014). However, few studies have examined the potential effects of projected future CO₂ levels on large pelagic fish. In the few studies conducted to date, there were no consistent effects of ≥2100 μatm CO₂ on early life history traits of cobia (Bignami et al., 2013) or dolphinfish (Bignami et al., 2014), but oxygen consumption and swimming ability were reduced in dolphin fish at >1400 μatm CO₂ (Bignami et al., 2014; Pimentel et al., 2014). Consequently, there appear to be different sensitivities to elevated CO₂ among large pelagic species. Bromhead et al. (2014) recently reported negative effects of elevated CO₂ on growth and survival of yellowfin tuna, but the effects were predominantly observed above 2100 μatm and were strongest and most consistent above 8500 μatm. There is clearly a need to test the sensitivity of other large pelagic fish to high CO₂, especially at CO₂ levels that are consistent with climate change projections for this century.

The yellowtail kingfish, Seriola lalandi, is a large pelagic fish found throughout the subtropical and temperate western Pacific, including New Zealand and Australia. Adults are mostly found in open coastal waters where they form schools associated with areas of high water flow near rocky outcrops or pinnacles (Kailola et al., 1993; Francis, 2012). Juveniles are generally found in offshore waters, often associated with drifting algae and debris (Kailola et al., 1993). Kingfish are piscivorous, feeding mostly on small pelagic fish (Francis, 2012). In New Zealand, they are a revered sports fish and form an important recreational fishery (ca. 600 t annual catch), with a small (ca. 170 t annual catch), but high value, commercial fishery also in operation (Ministry for Primary Industries, 2014).

The aim of this study was to conduct an initial assessment of the sensitivity of early life history stages of the yellowtail kingfish to elevated CO₂. We focused on the embryonic and pre-feeding larval stages as they may be more sensitive to environmental stress than later life stages (Menzel et al., 2009). Focusing on the earliest life stages also avoided problems associated with high and stochastic mortality of feeding-stage larvae in captivity. Eggs and larvae were reared for a total of 6 d, from 12 h post-fertilization to 3 dph. We used two elevated CO₂ treatments, one relevant to projections for the open ocean by year 2100 under emissions scenario RCP 8.5 (880 μatm CO₂) and a second, higher treatment (1700 μatm CO₂), relevant to CO₂ levels in high-productivity upwelling zones where pelagic fish often spawn (Bromhead et al., 2014). The higher CO₂ treatment also enabled a comparison with previous studies on pelagic and commercially important fish that have been conducted at ≥1600 μatm CO₂ (e.g. Frommel et al., 2012; Chambers et al., 2014; Pimentel et al., 2014). We investigated effects of elevated CO₂ on both life history traits and behaviour. For life history traits, we examined the effect of elevated CO₂ on hatching success, survival to 3 d post-hatch (dph), larval size, and yolk and oil globule development. For behavioural assays, we concentrated on three primary behavioural responses that can be affected by high CO₂ in small larval fish: activity level, startle response, and phototaxis.

Methods

Study location and fish husbandry

This study was conducted at the National Institute of Water and Atmospheric Research, Bream Bay Aquaculture Park, Ruakaka, Northland, New Zealand. Two sets of experiments were conducted in conjunction with mass spawning events that occurred during the nights of 26–27 January 2014 and 03–04 February 2014. The experiments from the two different spawning events are referred to as Trials 1 and 2. Larvae from each spawning were reared to 3 dph, which marks the end of the endogenous feeding stage (Moran et al. 2007; Martinez-Montano et al., 2014). A subset of larvae in Trial 1 were also reared to 6 dph without feeding to further explore growth on residual energy stores.
Broodstock, eggs, and larval culture
Spawning stock of yellowtail kingfish were maintained outdoors in 20 m³ circular tanks, each contained up to 10 locally sourced, wild-caught fish that had been domesticated in tanks for up to 6 years (approximately equal sex ratio in each tank). Each 20 m³ tank was supplied with 130 l min⁻¹ seawater filtered to 10 µm at ambient temperature (from 13°C in winter to 24°C in summer) and with an ambient photoperiod. Broodstock were fed a mixture of pilchard (Sardinops sagax) and squid (Notot干部 spp.). Spawning was allowed to occur naturally and to maximize genetic variation, eggs were collected from multiple broodstock tanks in even proportions. For Trial 1, three broodstock tanks containing a total of nine females and eight males contributed to spawning. For Trial 2, a different combination of three broodstock tanks containing a total of nine females and seven males contributed to spawning. Time of spawning was consistent across all tanks, occurring within the last 2 h of daylight.

Kingfish eggs were collected using an external egg collector as described by Moran et al. (2007), with a 500-µm mesh net to retain eggs from the surface overflow of each tank. An equal proportion of floating eggs from all contributing tanks were mixed, rinsed with oxygenated water flow for 5 min, and disinfected with Tosylchloramide (chloramine-T) at 50 ppm for 15 min. Eggs were then rinsed with water and then evenly distributed to 15 conical rearing tanks. Each 400 l rearing tank received flow-through seawater at 20–21°C with a photoperiod of 14 h light and 10 h dark and at a flow rate of 4 l min⁻¹. Gentle aeration was maintained within each tank with a weighted 4 mm airline. Eggs hatched 3 d after stocking and larvae were reared for a further 3 d on their endogenous reserves. Dead eggs, larvae, and egg shells were removed daily by draining from an outlet at the bottom of the rearing tank.

Experimental system and water chemistry
Seawater pumped from the ocean was filtered through mixed-media (sand), bag filtered to 5 µm, UV light treated to 150 mW cm⁻², and delivered to large a header tank. Oxygen diffusers in the header tank maintained baseline minimum dissolved oxygen (100% saturation) and a foam fractionator removed any additional organics. Seawater from the header tank was gravity-fed into three separate 100 l sump tanks where CO₂ was maintained at ambient control (≤450 µatm), mid (≤880 µatm), and high (≤1700 µatm) CO₂ (Table 1). Seawater from each of the three pH treatment sumps was pumped into five of the 400 l rearing tanks, so that there were five replicate tanks where CO₂ was maintained at ambient CO₂ level. A Hailea HX-6540 aquarium pump in the pH treatment sump both mixed the experimental seawater and a foam fractionator removed any additional organics. Seawater from each sump to the rearing tanks. The pHtotal (SG8 SevenGo pro, Mettler Toledo, Switzerland) and temperature (C22, Comark, Norwich, UK) of each rearing tank was measured daily and seawater CO₂ confirmed with a portable CO₂ equilibrator and an infrared sensor (GMP343, Väisälä, Helsinki, Finland). Tris buffers were obtained from Professor A.G. Dickson (Scripps Institution of Oceanography). Water samples were taken from three random tanks at each CO₂ level at the start and end of each trial. Water samples were analysed for total alkalinity by Gran titration (888 Titrand, Metrohm, Switzerland) to within 0.4% of certified reference material (Professor A.G. Dickson, Scripps Institution of Oceanography). Carbonate chemistry parameters were calculated in CO2SYS (http://cdiac.ornl.gov/ftp/co2sys/CO2SYS_calc_XLS_v2.1) using the constants K1 and K2 from Mehrbach et al. (1973) refit by Dickson and Millero (1987), and Dickson for KHCO₃. Seawater carbonate chemistry parameters for each trial are summarized in Table 1.

Sampling protocols for life history traits
Eggs were sampled at collection (~12 h post-fertilization) and larvae were sampled at hatching (0 dph) and 3 dph to estimate survival and to compare growth and development among CO₂ treatments. This involved mixing the eggs or larvae within each rearing tank using aeration and mechanical mixing with a hand-held agitator. Five samples of 520 ml were then taken with a beaker and eggs/larvae counted on a 500-µm mesh flat screen. The average of the five counts was used to calculate the total number of eggs or larvae in each rearing tank (using the sample volume to tank volume ratio).

Each rearing tank contained ~50 000 larvae. Morphometric traits were measured in a random subset of 20 fish sampled from each tank. We measured seven morphometric traits that are indicators of growth and performance in larval fish: standard length (SL), muscle depth at vent (MDV), total depth at vent including fins (TDV), yolk length (YL) and yolk depth (YD), oil globule diameter (OG), and eye diameter (ED) (Figure 1; Jones and McCormick 2002; Holt 2011; Chambers et al., 2014). Measurement landmarks followed Chambers et al. (2014). Each sampled larva was photographed with a Leica camera fitted to a Leica compound microscope. Morphometric traits were extracted from the photographs using ImageJ software with the image displayed on a high-resolution computer screen.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatment</th>
<th>CO₂ Level</th>
<th>Temperature (°C)</th>
<th>Salinity</th>
<th>pHtotal</th>
<th>Total alkalinity (µmol kg⁻¹ SW)</th>
<th>pCO₂ (µatm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>20.2 ± 0.1</td>
<td>34.6</td>
<td>7.99 ± 0.01</td>
<td>2330.3 ± 9.4</td>
<td>4688 ± 9.9</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>20.2 ± 0.1</td>
<td>34.6</td>
<td>7.77 ± 0.01</td>
<td>2331.9 ± 3.6</td>
<td>885.9 ± 23.3</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>High</td>
<td>20.3 ± 0.1</td>
<td>34.6</td>
<td>7.49 ± 0.01</td>
<td>2328.0 ± 8.5</td>
<td>1684.2 ± 64.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>20.5 ± 0.2</td>
<td>34.6</td>
<td>8.03 ± 0.02</td>
<td>2326.6 ± 5.9</td>
<td>427.5 ± 29.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>20.5 ± 0.2</td>
<td>34.6</td>
<td>7.76 ± 0.01</td>
<td>2328.1 ± 5.0</td>
<td>874.7 ± 17.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>High</td>
<td>20.5 ± 0.2</td>
<td>34.6</td>
<td>7.48 ± 0.02</td>
<td>2320.9 ± 4.3</td>
<td>1755.1 ± 73.6</td>
<td></td>
</tr>
</tbody>
</table>

Temperature, salinity, pHtotal and total alkalinity were measured directly. pCO₂ was estimated from these parameters in CO2SYS. Trial 1 used eggs from mass spawning on 26–27/01/2014 and Trial 2 used eggs from mass spawning on 03–04/02/2014.

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*Table 1. Mean (± s.d.) temperature, salinity, pHtotal, total alkalinity, and pCO₂ in experiments with yellowtail kingfish (S. lalandi) eggs and larvae.*
In Trial 1, we also conducted a pilot study where a subset of larvae was reared to 6 dph without feeding to further examine growth on remaining exogenous energy reserves. Owing to logistical constraints, only one rearing tank from each CO2 treatment could be continued to 6 dph. Thirty randomly selected larvae were sampled from each rearing tank as described for 3 dph. SL was the only morphometric trait measured in the 30 × 6 dph larvae sampled from each CO2 treatment.

**Behavioural assays**

All behavioural assays were conducted on larvae at 3 dph. Rearing tanks were mixed by aeration and a random sample of well-mixed larvae removed with beakers. Activity level and startle response assays were videographed from above with a Canon Powershot G15 digital camera placed on a tripod directly above the experimental arena. Larval behaviour was quantified subsequently from video playback. All assays were conducted in seawater at the same CO2 level as the experimental treatment of the individuals tested and seawater was changed after each assay.

Activity level: Activity levels were measured by gently transferring 10 individuals into a round plastic aquarium (14.5Ø × 9.2 H cm) with black sides and a clear base. Under the clear base, a white sheet of paper with thin black gridlines at 1 cm intervals provided a reference scale. The aquarium was filled with seawater to a depth of 2 cm (330 ml). The 10 larvae were videoed for 8 min. The first 5 min of the video recording provided a habituation period. Activity levels were assessed 5 min into the video recording for 2 min. The total number of line crosses on the 1-cm² grid made by the 10 individual larvae during this 2 min period was determined. Duplicate assays were conducted for each rearing tank.

Startle response: Groups of 10 larvae were treated exactly as described above except that a lead weight on a swinging pendulum was released after 8 min. The weight collided with the side of the aquarium and larval startle responses and behaviour were videoed for a further 1 min. Ten larvae were gently transferred using a beaker into the 5 × 15 cm habituation section and given 5 min to habituate. The LED light was then turned on and the plastic partition removed. The number of larvae in each third of the aquarium (dark, mid, and light) was recorded after 2 and 10 min. The phototaxis assay was repeated six times with different larvae from each rearing tank.

**Statistical analysis**

Repeated-measures (RM) ANOVA was used to compare survival to hatching and 3 dph among CO2 treatments. This analysis compared the estimated number of individuals in each rearing tank at the three developmental stages (fertilized eggs, hatched larvae, and 3 dph larvae) in Trials 1 and 2. CO2 treatment and trial were the independent variables and survival at developmental stage was the repeated measure.

Morphometric traits (SL, MDV, FDV, YL, YD, OG, and ED) were analysed for each of the 20 randomly selected fish per rearing tank. Morphometric traits were not highly correlated with each other (correlation coefficients between pairs of measures varied from

**Figure 1.** Photograph of 3 dph larval yellowtail kingfish (S. lalandi) showing morphometric traits measured. SL, standard length; MDV, muscle depth at vent; FDV, total depth at vent including fins; YL, yolk length; YD, yolk depth; OG, oil globule diameter; ED, eye diameter.
Effects of elevated CO2 on early life history development

0.10 to 0.58). Linear mixed-effects models (LME), with CO2 and trial as fixed effects, were used to determine any potential effect of elevated CO2 on morphometric traits. Tank was included as a random effect to account for the subsampling of fish from replicate tanks within each CO2 treatment. Individual fish were the level of replication \((n = 599)\). One deformed fish was excluded from the final analysis.

One-way ANOVA was used to compare SL among CO2 treatments in 6 dph larvae in the pilot study conducted in Trial 1. Individual fish were the level of replication \((n = 90)\). As there was one rearing tank per CO2 treatment in this analysis, the results must be interpreted with caution.

Factorial ANOVA was used to compare activity level among CO2 treatments and trials. Activity level was determined by summing the total number of line crosses of the 10 individuals in each behavioural assay. The average of the duplicate assays per rearing tank was then used in the analysis. Tank was the level of replication in this analysis \((n = 30)\).

Startle response was only tested in Trial 2. Logistic regression was used to compare the proportion of assays where the focal larva responded to the stimulus in the startle test vs. the proportion of assays where the focal larva did not respond to the stimulus. CO2 treatment was the categorical variable in the logistic regression. Because the size of the focal larva and their distance from the stimulus strike-point may influence the likelihood of a reaction, these two factors were included as covariates in the model. The inclusion of tank did not improve the fit of the model, so it was not included in the final model. To maximize statistical power, the two assays per rearing tank were used in the analysis. Assay was the level of replication in this analysis \((n = 30)\).

For fish that did respond to the stimulus \((n = 24)\), LME was used to determine any potential effect of CO2 on each of the three escape traits that were measured. CO2 treatment, fish size, and distance of the fish from the stimulus were included as independent fixed effects in the models and tank was included as a random effect. Individual fish were the level of replication in this analysis \((n = 24)\).

Phototaxis was only tested in Trial 2. LME was used to compare the proportion of larvae in the lit third of the chamber at 2 and 10 min among CO2 treatments. CO2 and time were the fixed effects, with rearing tank and assay container included in the model as random effects. Proportions were logit transformed before analysis. Assay was the level of replication in this analysis \((n = 90)\).

LME analysis was performed in TIBCO Spotfire S+ 8.2. All other analyses were conducted in Statistica (version 12).

**Results**

As expected, the number of surviving individuals per tank declined from fertilized eggs to hatching and 3 dph (Figure 2; RM ANOVA \(F_{2,52} = 81.89, P < 0.001\)). There was also a significant interaction between developmental stage and trial \(F_{2,52} = 10.16, P < 0.001\), with fewer eggs surviving to hatching in Trial 2 compared with Trial 1 (Figure 2). However, there was no effect of CO2 treatment on survival to hatching or survival to 3 dph.

Oil globule diameter was the only morphological trait that varied among CO2 treatments in 3 dph larvae. Mean oil globule diameter exhibited a decline with increasing CO2 level (Figure 3). Oil globule diameter was significantly smaller than the controls in the 1700-μatm treatment \((LME \tau_{26} = -2.14, P = 0.04)\), but not in the 880-μatm CO2 treatment \((LME \tau_{26} = -1.24, P = 0.23)\). However, the magnitude of the effect was relatively small, with oil globule diameter ~5% smaller in the 1700-μatm treatment compared with the control. SL, MDV, FDV, YL, YD, and ED did not differ among CO2 treatments for 3 dph fish. In the subset of fish that were reared to 6 dph in Trial 1, SL was ~2.5% smaller in the
two elevated CO2 treatments compared with the control (Figure 4; $F_{2,87} = 10.48$, $P < 0.001$), although this result must be interpreted with caution as there was no tank replication. Other morphological traits were not measured at 6 dph.

Activity level, measured as line crosses by 10 randomly selected individuals in a 2-min period, did not differ between CO2 treatment or trial. Average line (±SE) crosses were 100 ± 7.8 in the control, 80 ± 9.8 in 880 μatm CO2, and 116 ± 12.8 in the 1700 μatm CO2.

Eighty per cent of individuals (24 of 30) responded to the stimulus in the startle test. Distance from the stimulus ($\chi^2 = 4.39$, $P = 0.036$), but not CO2 treatment ($\chi^2 = 0.28$, $P = 0.869$), influenced the likelihood of responding to the stimulus. Among responders, there was no effect of CO2 treatment, or the covariates (fish size and distance from stimulus) on escape distance or escape duration among the CO2 treatments.

In the phototaxis experiment, more larvae were in the lit third of the chamber at 10 min compared with 2 min ($t_{87} = 5.07$, $P < 0.001$), but there was no effect of CO2 treatment.

**Discussion**

While an increasing number of studies have examined the effects of projected future CO2 levels and ocean acidification on marine fish, little is known about the effects on large pelagic fish. Here, we found that elevated CO2 influenced the utilization of endogenous energy resources of newly hatched yellowtail kingfish, but not their survival or behaviour. Our results suggest that the earliest life stages of yellowtail kingfish are likely to be tolerant to projected future CO2 levels in the ocean.

While some studies have reported negative effects of near-future CO2 levels on the growth of larval fish (e.g. Baumann et al., 2012), others have observed no effect (Bignami et al., 2013; Frommel et al., 2013; Hurst et al., 2013), or even increased growth rates (Munday et al., 2009a; Chambers et al., 2014; Pope et al., 2014). The reason for these contrasting findings is unknown, but they do indicate that the effects of elevated CO2 on larval development may differ greatly among taxa. We found no effect of elevated CO2 levels on growth or survival of larval kingfish to 3 dph. There was, however, a decline in oil globule size with an increasing CO2 level, which indicates that larvae utilized a larger proportion of their endogenous energy reserves in reaching the same size in high CO2 treatments. Oil globule diameter was 5% smaller in the 1700-μatm treatment, which would equate to an ~15% volume reduction in a spherical oil globule. While not a large effect, it could still have implications for individual performance. The oil globule of larval fish is a rich source of lipids that are catabolized during development (Heming and Buddington, 1988) and these lipids are the major sources of energy for growth and development after hatching (Rønnestad et al., 1998). The oil globule is also an important energy buffer in larval fish and may enhance resistance to starvation once larvae commence exogenous feeding (Chambers et al., 2009; Fisher et al., 2007). The increased use of this key energy source in reaching a similar size at first feeding in yellowtail kingfish could have implications for the growth and survival of older age classes. In particular, older larvae may be more susceptible to mortality at times or places where food availability is suboptimal.

Consistent with greater energetic demands in the elevated CO2 treatments, larvae reared for a further 3 d without feeding, to 6 dph, were slightly smaller (2.5% of SL) in the elevated CO2 treatments compared with control fish. The mixed-feeding period, immediately after the capacity for exogenous feeding is reached but when endogenous energy reserves are still being used, is a critical period for larval fish (Kamler, 1992). In particular, they are especially vulnerable to starvation during this stage. The slower growth of feeding-stage larvae on residual energy reserves is consistent with increased energetic demand in a high CO2 environment.

While we detected modest effects of high CO2 on energy utilization by larval kingfish, there were no direct effects of 880 or 1700 μatm CO2 on other morphometric traits or survival. This suggests that the development of embryos and newly hatched larvae of kingfish are tolerant to projected near-future CO2 levels, but there are some energetic costs to this tolerance. A decline in yolk sac area in larval fish exposed to high CO2, corresponding to an increase in body length, has been observed in larval clownfish (Munday et al., 2009a) and summer flounder (Chambers et al., 2014), indicating a different shift in energy utilization patterns in these species compared with the yellowtail kingfish studied here. In larval herring, yolk sac area was not affected by high CO2 levels, but there was a drop in RNA/DNA ratios, potentially indicating an increased energetic cost to larvae at elevated CO2 levels. Taken together, these studies suggest that there may be some energetic cost for larval fish to maintain growth and development in a high CO2 environment, but how larvae fuel this cost, or the trade-offs made in energy allocation to different physiological processes, may differ among species.

There was no effect of either CO2 treatment on swimming activity of the larvae, or the distance they travelled when startled. These results are consistent with the absence of any effect of 1800 μatm CO2 on swimming kinematics of larval cod, but contrast with studies on dolphinfish, where reduced swimming activity (Pimentel et al., 2014) and maximum velocity (Bignami et al., 2014) were observed at 1600 and 1460 μatm CO2, respectively. While it is possible that these different results could relate to differences in experimental design, they could also indicate that there are differences among pelagic fish in their sensitivity to high CO2. Furthermore, Bignami et al. (2014) observed an effect on swimming velocity in one experiment, but not another.

![Figure 4. Mean (± SE) standard length of yellowtail kingfish (S. lalandi) larvae from control, moderate, and high CO2 treatments at 6 dph in Trial 1. Other morphometric traits were not measured in 6 dph larvae. n = 30 larvae per treatment. Asterisk indicates statistically significant difference from control (P < 0.05). Note that the y-axis does not start at zero.](https://academic.oup.com/icesjms/article-abstract/73/3/641/2458924/646)
therefore, the effects may be highly dependent on developmental stage or other environmental factors. More studies using identical experimental protocols in a range of species would be helpful in identifying if the variable results reported to date are indicative of differences in sensitivity to near-future CO2 among species and/or developmental stages, or simply due to differences in experimental approaches.

Contrary to expectations, we did not detect significant effects of elevated CO2 on the behaviour of 3 dph larval kingfish. Larval and juvenile reef fish are more active (Munday et al., 2010) and have impaired responses to a threat stimulus (Allan et al., 2013) when reared at CO2 levels similar to those used here. Furthermore, newly hatched larval gobies are more positively phototactic if exposed to high CO2 during embryonic development (Forsgren et al., 2013). In contrast, there was no effect of high CO2 on activity, startle responses, or phototaxis in 3 dph yellowtail kingfish. This raises the question; why didn’t we detect behavioural effects in 3 dph larval kingfish when a wide range of behavioural and sensory impairments have been observed in reef fish? The behavioural changes observed in reef fish at high CO2 appear to be caused by an interference with the GABA-A receptor, the primary inhibitory neurotransmitter receptor in the vertebrate brain (Nilsson et al., 2012; Hamilton et al., 2014). The GABA-A receptor is an ion-channel with conductance for Cl\(^{-}\) and HCO\(_3\)\(^{-}\). Under normal conditions, ion gradients over the neuronal membrane result in an inflow of Cl\(^{-}\) and HCO\(_3\)\(^{-}\) upon opening of the GABA-A receptor, which then leads to hyperpolarization and inhibition of the neuron (Nilsson et al., 2012). However, fish excrete Cl\(^{-}\) and accumulate HCO\(_3\)\(^{-}\) from seawater to prevent an acidosis when exposed to elevated CO2 (Heuer and Grosell, 2014). Depending on the magnitude of changes in HCO\(_3\)\(^{-}\) and Cl\(^{-}\) during acid–base regulation, the resultant alterations of ion gradients could either potentiate the GABA-A receptor function, or reverse its action, making it excitatory rather than inhibitory (Nilsson et al., 2012; Hamilton et al., 2014). The absence of behavioural effects in 3 dph kingfish could indicate that HCO\(_3\)\(^{-}\) and/or Cl\(^{-}\) gradients have not changed sufficiently in these small preflexion fish to induce behavioural effects.

The mechanisms of acid–base regulation in embryonic and larval fish are very poorly understood (Brauner, 2008). That pH regulation occurs in these early life stages is clear, but the precise mechanisms and transporters involved are largely unknown. In juvenile and adult fish, the gills are the primary site of ion exchange for acid–base regulation. However, they cannot be involved in acid–base regulation of embryos or newly hatched larvae of pelagic fish, such as the S. lalandi larvae studied here, because they do not have gills. Instead, the skin and yolk epithelium are the primary site of ion exchange in the small larvae of broadcast spawned fish. Perhaps, kingfish larvae may be more reliant on Na\(^{+}\)/H\(^{+}\) exchange during early development and then shift to Cl\(^{-}\)/HCO\(_3\)\(^{-}\) as the primary mechanism for acid–base regulation when the gills develop, which occurs between 2 and 15 dph (Martinez-Montano et al., 2014). An increased dependence on Na\(^{+}\)/H\(^{+}\) exchange across the skin and yolk sac before the development of gills could explain the absence of behavioural effects in 3 dph yellowtail kingfish. A greater reliance on Na\(^{+}\)/H\(^{+}\) exchange during early ontogeny would mean that Cl\(^{-}\) concentrations may not decline as a result of acid–base regulation (Hayashi et al., 2004). The maintenance of Cl\(^{-}\) concentrations may be sufficient to maintain normal function of the GABA-A receptors in a high CO2 environment. Alternatively, the GABA-A neuroreceptors of early developmental stages may be far less sensitive to the change in ion gradients than they are in older and more developed fish. There is currently insufficient knowledge about the acid–base regulatory processes in the early developmental stages of marine fish, and the mechanism by which high CO2 levels interfere with neurotransmitter function, to properly assess this hypothesis, but it would be an interesting avenue for future investigation.

Frommel et al. (2012) observed tissue damage in Atlantic cod larvae at 32 dph, which coincided with metamorphosis and gill development. However, tissue damage was not evident in older larvae. Similar patterns of tissue damage in early larval stages have been observed in anchovies (Frommel et al., 2014) and summer flounder (Chambers et al., 2014), both of which also hatch as very small larvae without functional gills and metamorphose after 3–4 weeks. Consequently, the shift from ion regulation across the skin and yolk sac to the gills may be a critical time in the development of broadcast spawned fish (Frommel et al., 2012). In contrast, the larvae of demersally spawned reef fish are relatively large at hatching and develop quickly during the larval stage. Consequently, there could be very different effects of high CO2 on the small and slowly developing larvae of temperate water broadcast spawning fish compared with the relatively large and fast developing larvae of tropical benthic spawning fish. Owing to the delayed development of gills, accompanied by branchial exchange of Cl\(^{-}\)/HCO\(_3\)\(^{-}\) for acid–base regulation, temperate water broadcast spawning fish may be more prone to morphological and energetic effects of high CO2 during early life stages. In contrast, the large size and rapid development of gills may predispose tropical benthic spawned fish to behavioural effects of high CO2. This hypothesis deserves further exploration and testing.

As with all ocean acidification research, any effects of high CO2 observed in experimental studies must be considered in the context of the rate of environmental change and the potential for organisms to adapt (Sunday et al., 2014). Perhaps the modest effects of high CO2 on energy provisioning observed here in larval kingfish could be ameliorated by adaptation as CO2 levels slowly rise in the ocean over coming decades. Furthermore, recent studies have shown that parental exposure to high CO2 can reduce the effect of high CO2 on larval and juvenile growth and survival (Miller et al., 2012; Murray et al., 2014). In this initial study of the effects of projected future CO2 levels on the early developmental stages of larval kingfish, it was not possible to test for adaptive responses through evolution or plasticity; however, this should be a priority for future studies.

In conclusion, this study indicates that the pre-feeding larval stages of yellowtail kingfish are tolerant to projected near-future CO2 levels, but there could be an energetic cost to this tolerance. Consequently, larvae may have lower energy reserves at the onset of feeding in a future high CO2 world. Contrary to expectations, we observed no significant effects of high CO2 on larval behaviour. We propose that behavioural effects of high CO2 may not occur until larvae begin Cl\(^{-}\)/HCO\(_3\)\(^{-}\) acid–base regulation when the gills develop; however, further studies are required to test this hypothesis. A prediction from this hypothesis is that the small larvae of broadcast spawned fish, that do not have functional gills until flexion, may be less susceptible to behavioural effects of high CO2 than the relatively large larvae of demersally spawned fish. Nevertheless, later ontogenetic stages may still suffer behavioural impairment. Future studies should test how spawning mode and developmental stage influences the sensitivity of larval fish to the effect of high CO2 and ocean acidification.
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Effects of elevated CO2 on early life history development