

Ocean acidification impacts upon tropical tuna populations

A full project proposal submitted to the PFRP by the Secretariat of the Pacific Community (SPC) and the Inter-American Tropical Tuna Commission (IATTC)

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1. Summary

The unaccounted impacts of ocean acidification (and warming) upon tuna stocks in the Pacific (and globally) represent a serious risk to the achievement of sustainability based management objectives for both Regional Fisheries Management Organisations (RFMOS) and for the policies of sovereign states responsible for tuna fisheries management in the Pacific region. Research has demonstrated that the early life history stages of some fish species (and numerous other marine organisms) are sensitive to ocean acidification levels that are projected to occur by the end of this century. Those findings have significant implications for future recruitment success and population levels for those species. Utilising the long established expertise and unique facilities at the IATTC's Achotines Laboratory in Panama, the first year of this project aims to elucidate the impacts of projected ocean acidification levels upon processes and life history stages of yellowfin tuna (*Thunnus albacores*) that are considered critical to recruitment success: sperm motility, fertilisation rates, embryonic development, hatching rates, condition, development, growth and survival in pre- and post-feeding larvae. Empirical results from the laboratory trials will then be used, in conjunction with physical oceanographic data from ocean acidification projection models, to parameterise the SEAPODYM model and evaluate the impact of ocean acidification upon the distribution and abundance of yellowfin tuna in the Pacific Ocean. The outputs from this project will reduce uncertainty regarding future stock trends as provided to tuna RFMOs in the Pacific, increasing the likelihood that these organisations can make decisions that ultimately achieve sustainability based management objectives.

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4. Research Plan

4.1 Background and justification

The Pacific tuna fisheries are among the largest, most complex and valuable fisheries resources in the world. In 2008, the most recent year with confirmed statistics, the total tuna catch exceeded 3 million tonnes (Williams and Terawasi, 2009; IATTC, 2009), comprised 70% of the total global tuna catch and was valued at USD 4.8 billion dollars. The Western and Central Pacific Fisheries Commission (WCPFC), the Inter-American Tropical Tuna Commission (IATTC) and the Western Pacific Regional Fisheries Management Council (WPFMC) are responsible for ensuring the sustainable use of this globally significant resource.

In managing these fisheries, these bodies are guided by their respective Conventions, and in particular, objectives within these to manage the catches to attain but not exceed the maximum sustainable yield (MSY). Conservation and Management Measures (e.g. WCPFC CMM 2008-01) and resolutions (e.g. IATTC Resolution C-09-01) are designed to ensure these primary objectives are met.

In designing management strategies to meet these objectives, quantitative stock assessments and model based projections are used to allow consideration of the potential future responses of the tuna stocks to alternate management scenarios. While it is recognised that changing environmental processes can significantly influence population processes (recruitment, growth, natural mortality), current assessment models and projections rarely explicitly include such effects due to a lack of information on their impacts. There is now, however, conclusive scientific evidence that two significant and anthropogenically forced long-term shifts in global ocean conditions are currently in progress and will continue into the future, these being ocean warming (e.g. Barnett et al. 2005) and ocean acidification (Caldeira and Wickett, 2003; Feely et al. 2004). Both temperature and pH are environmental factors that can influence the growth, condition and survival of fish (e.g. Buckley et al. 1990; Ishimatsu et al. 2004) and therefore affect population dynamics and abundance (Pepin, 1991).

The ecosystem model SEAPODYM is structured to evaluate the impact of such environmental changes (Lehodey et al 2008). It explicitly integrates physical oceanography with forage and tuna population dynamics to spatially predict the distribution and abundance of tuna (Lehodey et al 2008). The model is currently being used to forecast the changes in tuna distribution and biomass associated with the current IPCC scenarios for ocean warming in the Pacific Ocean (Lehodey et al, 2010).

Unfortunately, no consideration has been given to the potential impacts of ocean *acidification* upon the target tuna populations in the Pacific, despite growing evidence that it is likely to have a very profound impact upon many marine species populations and ecosystems by the end of this century and may threaten some of the world's most productive fisheries (Guinotte and Fabry, 2008). SEAPODYM could be applied to also forecast the impacts of ocean acidification on tuna resources in the Pacific if information on the effects of pH on population dynamics of tuna can be collected.

What is ocean acidification?

Oceanic waters show both spatial and temporal variation in pH, with surface waters being slightly alkaline (average pH 8.2) and varying by about 0.3 units due to physical and biological oceanographic processes (Raven et al., 2005). A number of factors influence oceanic uptake or release of CO₂ from surface waters, including atmospheric CO₂ concentrations, sea surface temperature, biological (primary) production, and physical oceanographic processes such as upwelling of CO₂ rich waters.

Ocean acidification is defined as the “change in ocean chemistry driven by the oceanic uptake of chemical inputs to the atmosphere, including carbon, nitrogen, and sulfur compounds” (Guinotte and Fabry, 2008). Anthropogenic atmospheric CO₂ is by far the largest contributor to ocean acidification today, with an estimated 30-50% of global anthropogenic CO₂ emissions absorbed by the world's oceans (Feely et al 2004, Sabine et al. 2004, Orr et al 2005). The net effect of adding CO₂ to seawater is to increase concentrations of H₂CO₃ (carbonic acid), HCO₃⁻ (bicarbonate ion) and H⁺ (hydrogen ions) and decrease concentrations of CO₃²⁻ (carbonate ions) and lower pH (Fabry et al 2008). Hence, the increased CO₂ absorption has led to a 30% increase in oceanic hydrogen ion (H⁺) concentration and a reduction in pH (increased ocean acidity) by 0.1 units since the start of the industrial revolution

(Caldeira and Wickett, 2003) as well as a reduction in the number of carbonate ions (CO_3^{2-}) available. It is estimated that this future uptake of atmospheric CO_2 by global oceans will further reduce the pH of oceanic surface waters by an average 0.3-0.4 units by 2100 (Caldeira and Wickett, 2003, 2005), with larger reductions predicted at higher latitudes (Ilyina et al. 2009b). These reductions in the pH of ocean surface waters represent shifts in pH larger than any thought to have occurred in millions of years, outside of rare catastrophic events (Feely et al. 2004). The predicted rate of change is very fast relative to past rates of change.

Potential impacts of ocean acidification upon tuna populations

Changes in water chemistry associated with increased CO_2 are expected to impact upon tuna populations, and this sensitivity is expected to vary depending on the specific developmental stage. For productive species like tuna, the rate of survival typically increases as fish develop and mature, and the environmental processes that significantly affect recruitment numbers act primarily upon the early life history stages (Pepin, 1991; Ferron and Leggett, 1994). Only a small proportion of larvae survive through to adulthood, with the consequence that even a tiny change in the mortality rate during these stages can have orders of magnitude greater effect on the number of recruits added to the adult population (Pepin, 1991; Buckley et al., 1999). Small changes have large implications for fisheries and their ability to gain maximum yields from stock while not compromising the reproductive capacity of the populations.

In the only known relevant experiment involving tuna, Kikkawa and colleagues (2003) found that 100% mortality of eggs of Eastern little tuna (*Euthynnus affinis*) occurred within 24 hours of exposure to elevated pCO_2 levels. However, the levels tested far exceeded those predicted using IPCC scenarios and these results cannot be used to confidently imply responses under projected ocean acidification levels.

However, concern over the *potential* impacts of ocean acidification on tuna populations arises from the rapidly growing body of evidence indicating significant negative effects on the physiology, growth and survival of a diverse range of other fish and marine organisms, in response to elevated CO_2 levels (Fabry et al. 2008; Guinotte and Fabry, 2008; Raven et al., 2005). There are two main mechanisms by which elevated pCO_2 and associated changes in ocean chemistry (including decreased pH) adversely impact marine organisms, these being via decreased carbonate saturation state, which directly affects calcification rates, and via disturbance to acid-base physiology (Fabry et al. 2008; Guinotte and Fabry, 2008). Indirect impacts upon a given organism or population are also expected to occur due to direct impacts upon other species within the ecosystems they are part of.

The reduction in the availability of carbonate ions (reduced carbonate saturation) due to increased CO_2 and lowered pH (Feely et al. 2004) makes calcification processes much more difficult for organisms which build skeletons, shells or other structures out of CaCO_3 , and in undersaturated waters, dissolution of CaCO_3 is favoured (Guinotte and Fabry, 2008). Decreased calcification in response to the reduced carbonate saturation state has been demonstrated in numerous species of coral, coccolithophores, molluscs, echinoderms and other calcifying organisms (e.g. Raven et al., 2005).

However, little attention has been given to the potential impacts on the development/growth of fish otoliths, which play a critical role in fish balance and hearing, and which are in large part comprised of aragonite, a form of calcium carbonate. The only known study, that of Checkley et al (2009), surprisingly found that larval white sea bass, grown under elevated CO_2 conditions, produced significantly *larger* otoliths compared to fish of the same size/age grown under control conditions. However, the impact of abnormally large otoliths (for size) upon fish condition and survival is still unknown (Checkley et al., 2009) and the impact of ocean acidification on otolith formation may depend on species specific capacity for acid-base regulation in the tissues surrounding the otoliths (Fabry et al 2008). Given the importance of otoliths to fish movement and behaviour determining potential impacts on tuna is a priority.

Acid base metabolism is another key area of potential impact in tuna. Increased partial pressure of CO_2 in the blood (hypercapnia) due to increased environmental pCO_2 , can induce acidosis in body tissues and fluids of larger marine organisms including fish (Portner et al., 2004), with evidence that

this can impact on numerous processes including the oxygen carrying capacity of blood (Portner and Reipschlager, 1996), protein synthesis and growth (Langerbuch and Portner, 2003) and reproduction (Portner et al, 2004). Such impacts are also likely to vary between developmental stages.

Unfortunately, studies on fish species which attempt to examine the likely impacts of ocean acidification on fish metabolism are relatively few. Many studies have tested for the potential effects of CO₂ levels which are much higher than projected under IPCC scenarios (Fabry et al 2008). Those studies have typically found very high mortality levels (Kikkawa et al., 2006; Hayashi et al. 2004), including in early developmental stages (eggs, larva) (Kikkawa et al. 2003, Ishimatsu et al. 2005) and other effects including reduced cardiac output (Ishimatsu et al. 2004) and metabolic capacity (Michaelidis et al. 2007). However, for such information to be relevant to the management of fisheries, they clearly need to be conducted through simulating the ocean chemistry conditions (CO₂ and pH levels) that are reflective of those predicted using the IPCC scenarios. Only very recently has research been presented (to the International Symposium on “*Climate Change Effects on Fish and Fisheries: Forecasting Impacts, Assessing Ecosystem Responses, and Evaluating Management Strategies*”, 25-29 April 2010) on ocean acidification impacts on larval fish using IPCC projections and these results are as yet unpublished in scientific journals, but suggest that susceptibility to ocean acidification will be species specific (Hollowed et al, 2010 and symposium abstracts therein).

Evidence has also been found for impacts of ocean acidification upon processes critical to reproductive success in marine organisms, namely gamete viability, fertilisation rates and embryonic development. Havenhand and colleagues (2008) tested the response of sea urchin gametes and larvae to seawater with CO₂ reduced pH by -0.4, (the upper limit of projected change by 2100) and found statistically significant reductions in sperm swimming speed and percent sperm motility and fertilisation rates (down 24%) and subsequent embryonic development. This study emphasises the importance of using CO₂ and pH levels predicted using IPCC scenarios and its findings suggest that it will be critically important to examine gamete and fertilisation sensitivities in other broadcast spawning marine species (including tuna) for which sperm are exposed to environmental pH prior to egg fertilisation. Similar effects on sperm activation and motility were found for white sturgeon (Ingerman et al. 2002), further emphasising the potential effects in fish. Similar effects on fertilisation and sperm motility have been found in other studies of sea urchin (Kurihara et al 2004).

Many environmental factors are thought to contribute to recruitment variation including water temperature (e.g. Buckley et al 1990), food availability/starvation (e.g. Lett and Kohler, 1976) and predation (e.g. Leggett and Deblois, 1994). The synergistic impacts of pH in combination with other environmental stressors likely to be encountered in the future (increased temperature) and encountered normally (starvation) may better define the likely direct impacts of ocean acidification upon tuna recruitment.

International concern

Recognition of and concern over the potential for recent, rapid changes in ocean chemistry to severely affect marine organisms, food webs, biodiversity, and fisheries within the next 50 years, led to the signing of the Monaco Declaration (2008) by 155 world leading ocean acidification researchers from 26 countries. The declaration states that ocean acidification is underway, is accelerating and severe damages to marine ecosystems are imminent and could “*affect marine food webs and lead to substantial changes in commercial fish stocks, threatening protein supply and food security for millions of people as well as the multi-billion dollar fishing industry*”.

This international concern is now also being reflected in government policy. The US Congress requested that the National Research Council conduct a study on ocean acidification in the *Magnuson-Stevens Fishery Conservation and Management Reauthorization Act of 2006* to assist federal agencies develop their understanding of and ability to address the issue. Shortly after the study was underway, Congress passed another law—the Federal Ocean Acidification Research and Monitoring (FOARAM) Act of 2009—which calls for, among other things, the establishment of a federal ocean acidification program (NRC, 2010). This report “*Ocean Acidification: A National Strategy to Meet the Challenges of a Changing Ocean*” was presented to a US Senate Committee Hearing on April 22nd 2010, with the report concluding that “*Present knowledge is insufficient to guide federal and state agencies in*

evaluating potential impacts for management purposes” and identifying an urgent need for Federally funded research into the impacts of ocean acidification, specifically focusing on 8 key areas including: Understand the physiological mechanisms of biological responses; Investigate the response of individuals, populations, and communities; Understand ecosystem-level consequences; Investigate the interactive effects of multiple stressors; Understand the socioeconomic impacts and informing decisions.

Justification

The unaccounted impacts of ocean acidification (and warming) upon tuna stocks in the Pacific (and globally) represent a serious risk to the achievement of sustainability based management objectives for both Regional Fisheries Management Organisations and for the policies of sovereign states responsible for fisheries management in the Pacific region. The higher risk expected for early life history stages and the observations from other marine species suggest that initial efforts to elucidate the impacts of projected ocean acidification upon tuna populations should focus on processes and stages that are critical to recruitment success: gamete impacts, fertilisation rates, embryonic development, hatching rates, condition, development, growth and survival in pre and post feeding larvae. To understand the implications for tuna population dynamics and for tuna fisheries, these potential effects need to be tested and taken into account in the population models currently used to evaluate stock dynamics in the Pacific Ocean. Yellowfin tuna has been chosen as the initial subject of these investigations, given that the required facilities and expertise to conduct these experiments for yellowfin tuna already exist and it is a key target species in both the WCPO and EPO.

4.2 Objectives

The objectives of this project are three-fold:

1. Firstly, to collect and collate the experimental data necessary to evaluate the potential impacts of altered gamete, fertilisation, embryonic development, hatching and larvae ecology from projected increases in ocean acidity in the Pacific Ocean for yellowfin tuna.
2. Secondly, to utilize the experimental data to parameterise the SEAPODYM model to evaluate the impact of projected ocean acidification (and warming) upon the distribution and abundance of yellowfin tuna.
3. Thirdly, provide information on the potential impacts of ocean acidification upon tuna stocks, in particular yellowfin tuna, to RFMOs for consideration in management decision making processes.

4.3 Methodology

4.3.1 Laboratory trials

The effects of ocean water acidification upon yellowfin tuna early life history processes and stages will be tested experimentally at the Achatines Laboratory in four phases:

1. Sperm and Fertilization trials
2. Egg/embryonic development trial
3. Yolk-sac larval stage trial
4. Early feeding larval stage trial

Based on the availability of 15 experimental tanks, 5 pH treatment levels will be used for all trials, with three replicate tanks per treatment. The treatment levels will be based on the outputs from the latest ocean carbon cycle models using IPCC IS92a Scenario (e.g. the HAMMOC model, as in Ilyina et al., 2009a, b) with the upper limit to be set as the highest sea surface pH level observed or predicted in the Pacific between now and 2200 (currently ~pH 8.2), the lower limit set as the lowest sea surface pH observed or predicted in the Pacific over that period (currently 7.8, but expected to be around 0.77 lower by 2200 according to Caldeira and Wickett, 2003, so around 7.0). The three remaining treatments will be set at intervals between the upper and lower limits, allowing a response curve for the primary larval response variable (mortality) to be modeled and fed into the SEAPODYM

spawning habitat index. One of the intermediate treatment levels will be a "control", with pH equal to the pH of the waters off Achotines (pH~7.9) from which the parent fish are originally obtained. So preliminary pH levels are likely to be: 8.2,7.9,7.6, 7.3,7.0 but may be adjusted if updated ocean carbon models become available between now and the trial's start date. Dr Tatiana Ilyina at the University of Hawaii currently uses the HAMMOC model to run ocean acidification projections (e.g. see Ilyina et al., 2009) and as a collaborator on this project, will be providing advice on the final treatment levels selected, according to the latest available model projections.

The four lower treatment levels of seawater pH will be maintained by regulation of mixtures of compressed air and carbon dioxide (CO₂) bubbled through air diffusers in each tank. The use of CO₂ is critical to modifying water chemistry (e.g. increasing carbonic acid, increasing H⁺, lowering pH) in a manner which is consistent with CO₂ induced ocean acidification. The highest pH level will be maintained by additions of dissolved sodium hydroxide to makeup water added to each tank. In all trials, water quality parameters (pH, temperature, salinity, dissolved oxygen, CO₂, alkalinity) will be measured at 2-hr intervals in each tank. For trials 2-4, 20 eggs/larvae will be sampled from each tank at the start and termination of the trials, fixed in 95% US grade ethanol and stored for subsequent morphometric analyses (size, shape) of the otoliths by high magnification light microscopy, to assess the potential impact of pH on otolith formation (e.g. Checkley et al 2009).

It should be noted that the protocols used for running trials 2-4 are very well established at Achotines and the risk of not obtaining usable results is considered very low. Trial 1 will be applying methods not previously used at Achotines for yellowfin tuna and is considered to contain a relatively higher risk. To counter this, we have developed a number of alternate strategies to reduce that risk.

4.3.1.1 Sperm activity and fertilization trials

A number of options have been identified from which to source the gametes required for this trial. Having these options available will be important as there is some risk that isolating gametes prior to fertilization in tank spawning yellowfin may be difficult. Hence the following additional options will be held in reserve, should this first option not be possible:

1. Strip spawning wild caught YFT (risk: capture of mature YFT off Achotines is less common than for black skipjack and while it may be possible to find ripe and running males during the day there may only be a small window in the afternoon or evening to capture females that have recently hydrated eggs).
2. Strip spawning wild caught black skipjack (mature individuals are available and can be captured off Achotines for much of the year). While it is probably possible to find ripe and running males during the day there may only be a small window in the afternoon or evening to capture females that have recently hydrated eggs. These results could not be applied in the yellowfin SEAPODYM model but would be invaluable for understanding potential impacts of acidification upon this vital reproductive process in tunas more generally.
3. Isolation of eggs and sperm from tank spawning black skipjack. This will require the establishment of broodstock black skipjack, which has been done in the past at Achotines Laboratory, and may require sacrifice of the male fish, to ensure isolation of sperm.

In each case, isolated gametes would be stored on ice (if transporting back from sea) and at 4C in the lab, with trials to be run within 24 hours. The male sperm would be divided, one part for use in the sperm activity trial, and the other for the fertilization trial.

15 conical, 250-L-volume tanks will be used to create 5 seawater pH treatments, each with 3 replicate tanks (5 x 3 = 15). A subsample of this will be removed and put aside on ice. The rest will be stored at 4C. 20mL of seawater from each tank will be pipetted into a separate test tube, with these randomly labeled 1-15 (not by the "reader" or technician responsible for taking the subsequent sperm activity counts) with the tube number and corresponding pH and tank number recorded on paper but not provided to the reader. The "reader" will then sequentially aliquot a uniform amount of semen into each test tube and gently mix to suspend. Using a high magnification light microscope, sub-samples will be transferred to a slide and counts of % sperm activity (e.g. Ingerman et al 2002) will then be

taken at 1 minute intervals, for each tube, until 0% activity is observed. This timing may be adjusted depending on the apparent mortality rate for an initial test trial using a known pH 8.1. This trial will allow a relative sperm activity curve to be generated for each treatment pH.

Unfertilised eggs will be stocked in each tank at a density of 80/L. Sperm recently stored at 4C will be brought up to room temperature. A test tube of seawater will be removed from each tank, and uniform samples of semen gently suspended in each and immediately mixed back into the tank. Number and percentage of eggs fertilized will be determined after 24 hours.

4.3.1.2 Fertilized egg trials

The effects of ocean water pH on fertilized eggs of yellowfin will be tested in replicated laboratory trials. For each trial, fertilized eggs will be collected from a daily spawn in the broodstock tank of the Achotines Laboratory and randomly stocked in each of 15 conical, 250-L-volume tanks. Eggs will be stocked in each tank at a density of 80/L. In each egg trial, each seawater pH treatment level will be replicated in 3 tanks (5 treatment levels x 3 replicates/treatment = 15 tanks). Egg trials will be repeated 3 times, each trial with eggs from different spawns, to minimize effects of variable egg quality from individual spawning events.

Each egg trial will continue until the point of 100% hatching (approximately 18 to 30 hrs depending on water temperature, Margulies et al. 2007a). The timing of 100% hatching in each tank will be determined by sequential sampling of eggs in each tank, beginning at 18 hrs after fertilization. One hour after 100% hatching, three 300-mL aliquot samples will be taken from each tank to determine % hatch. The % hatch will be determined for each sample as:

$\% \text{ hatch} = (\text{number of live larvae} / \text{number of live and dead larvae} + \text{number of unhatched eggs}) \times 100$

At the time of stocking, 30 eggs will be sampled fresh from each tank, measured, and processed for dry weight determination. At the end of each egg trial, 30 hatched larvae will be sampled fresh from each tank, measured (total length, notochord length, yolk volume, oil globule volume), and processed for dry weight determination (Margulies et al 2007a). At 4-hr intervals, 5 eggs/tank will be sampled and fixed for subsequent histological examination of tissue and organ development. At 2-hr intervals, water quality parameters (pH, temperature, salinity, dissolved oxygen, CO₂, alkalinity) will be measured in each tank

4.3.1.3 Yolk-sac larval trials

The effects of ocean water pH on yolk-sac larvae of yellowfin will be tested in replicated laboratory trials. For each trial, yolk-sac larvae will be collected 1 hr after hatching and randomly stocked in each of 15 circular, 720-L-volume, fiberglass tanks at a density of 15 larvae/L. In each yolk-sac larval trial, each seawater pH treatment level will be replicated in 3 tanks (5 treatment levels x 3 replicates/treatment = 15 tanks). Yolk-sac larval trials will be repeated twice, each trial with larvae from different spawns, to minimize the effects of variable larval quality from individual spawning events. Each yolk-sac larval trial will continue until just prior to larval eye pigmentation and mouth development (approximately 50-70 hrs after hatching depending on water temperature). Rearing experience by IATTC scientists at the Achotines Laboratory has shown that yellowfin larvae begin to develop eye pigmentation and mouth formation during the nighttime hours, approximately 44 to 55 hrs after hatching, just prior to first-feeding (which occurs at first-light the following morning) (Margulies et al 2007b). Rearing experience has shown that yellowfin larvae are randomly distributed in tanks throughout the yolk-sac period, up until the time of first-light coinciding with first-feeding. Once the first-feeding stage begins, larvae distribute themselves non-randomly in the tanks. Therefore, to accurately estimate density and % survival near the end of the yolk-sac phase, it is necessary to sample larvae during the nighttime while they are still uniformly distributed throughout the water column in the tanks.

During the nighttime period just prior to first-feeding, larvae will be sampled by 2.5-L-volume PVC “slurp” samplers, with 4-5 replicate “slurp” samples taken per tank. Mean larval density and % expected survival (adjusted for sample removals) (Margulies 1989) will be estimated for each tank.

At the time of stocking, and at the end of each yolk-sac trial, 30 larvae will be sampled fresh from each tank, measured (total length, notochord length, yolk volume, oil globule volume), and processed for dry weight determination. At 12-hr intervals, 5 larvae/tank will be sampled and fixed for subsequent histological examination of tissue and organ development. At 2-hr intervals, water quality parameters (pH, temperature, salinity, dissolved oxygen, CO₂, alkalinity) will be measured in each tank.

4.3.1.4 First-feeding larval trials

The effects of ocean water pH on first-feeding larvae of yellowfin will be tested in replicated laboratory trials. For each trial, late yolk-sac-stage larvae will be collected from incubation tanks and randomly stocked in each of 15 circular, 720-L-volume, fiberglass tanks at a density of 20 larvae/L. In each larval trial, each seawater pH treatment level will be replicated in 3 tanks (5 treatment levels x 3 replicates/treatment = 15 tanks). First-feeding larval trials will be repeated twice, each trial with larvae from different spawns, to minimize the effects of variable larval quality from individual spawning events. Each larval feeding trial will be conducted for 5-7 days (depending on overall survival trends). Larvae will be fed cultured rotifers (*Brachionus plicatilis*) at densities of 3-5/mL, and dense blooms of unicellular algae (750,000-1,000,000 cells/mL) (“green water”) will be maintained in each tank to facilitate rearing (IATTC rearing experience has shown that yellowfin larvae require green water to feed in laboratory tanks, Margulies et al 2007b).

After 5-7 days of feeding, each tank will be slowly drained of water, and all surviving larvae will be removed by beaker and counted. The % expected survival (adjusted for sample removals) will be estimated for each tank.

At the time of stocking, after 3 days of feeding, and at the end of the experiment, 30 larvae will be sampled fresh from each tank, measured (total length, notochord length, body depth at pectoral, body depth at vent), and processed for dry weight determination. At 24-hr intervals, 5 larvae/tank will be sampled and fixed for subsequent histological examination of tissue and organ development (including swim bladder inflation), and additionally, 10 larvae/tank will be sampled and fixed in 5% formalin for subsequent gut analysis (Margulies et al 2001). At 2-hr intervals, water quality parameters (pH, temperature, salinity, dissolved oxygen, CO₂, alkalinity) will be measured in each tank.

4.3.2 Data analysis

Data collected from the laboratory trials will be analyzed using generalized linear models (GLMs) with the statistical software R. A flexible approach will be taken to model the features observed in the data. Hatching rate and survival rate will be analyzed as binomial response variables, as will other rate estimates, such as incidence of swim bladder inflation. Morphometrics and dry weights of eggs and larvae, and final larval biomass per tank, will be analyzed as normally distributed response variables, and alternative distributions will be considered depending on residual patterns. Growth in dry weight and length will be analyzed using non-linear growth models. Analyses of numbers of prey/gut/larva will assume a negative binomial distribution. In cases where multiple samples (aliquots or slurp samples) have been used to estimate responses per tank, the observed response per sample will be modeled, in order to estimate the treatment effect.

4.3.3 SEAPODYM modeling

Physical and biogeochemical conditions influence tuna population dynamics through changes in spawning conditions, habitat suitability, and distributions of food resources that result in changes in fish movement behavior, reproduction and mortality. Environmental data are used in SEAPODYM to functionally characterize the habitat of the population depending on its thermal biogeochemical and forage preferences (Lehodey et al. 2008).

There are three types of habitat indices in the model: 1) thermal, 2) feeding and 3) spawning. These indices are used to control population dynamical processes (both spatial and temporal) such as movement to the feeding or spawning grounds, natural mortality and predation.

To simulate the predicted changes in ocean acidity in SEAPODYM, the spawning habitat index will be altered to include the effect of ocean acidity based on the results of the laboratory studies. A simple

response function to pH will be derived and parameterized from the laboratory work described in sections 4.3.1.1 to 4.3.1.4. In the model the spawning habitat is used to both scale the spawning success and to modulate the average natural mortality coefficient of larvae in space and time. This average mortality coefficient is estimated first in the model through data assimilation techniques (Senina et al 2008; Lehodey et al., 2010). Thus, the impact in larvae recruitment due to change in ocean acidity will be projected using the environmental forcing fields (temperature, currents, primary production, dissolved oxygen concentration and pH) from a Earth Climate simulation under a IPCC scenario (Ilyina et al., 2009a).

The spawning index in the model already accounts for several mechanisms, i.e. optimal spawning temperature and larvae prey-predator tradeoff (Lehodey et al. 2008). Thus, adding the new pH effect will not provide necessarily a linear response but instead can amplify or conversely compensate the other effects.

4.4 Expected Outcomes

The research plan is based around a two-year time line. The expected outcome at the end of two years is a model (SEAPODYM) based evaluation of the expected impact of ocean acidification upon the distribution and abundance of yellowfin tuna in the Pacific Ocean. The results from this modeling will be written up and reported to the Scientific Committees (or equivalent) of the WCPFC, IATTC and WPFMC. This information will enhance the capacity of these RFMOs to make more informed decisions regarding the management of the tuna resources, particularly with regard to attaining key sustainability related objectives.

4.5 Timing

This is a two year project, with laboratory based work to be completed in the first year (2011) and SEAPODYM modeling component to be completed in the second year (2012). Asterisks (**) below denote activities that would be part or fully PFRP funded. Outputs are highlighted in ***bold italics***.

Timing	Activity and Output
Oct - Dec 2010	Finalize physical experimental designs, compile list of needed materials and equipment as well as sources for purchasing and quotations.
Jan - Mar 2011	Purchase materials and equipment and ship to Achotines Laboratory, assembly and testing of physical experimental setups.(**)
Apr - May 2011	Laboratory trials (**)
Jul or Aug 2011	Tentative schedule for repetition or modification of laboratory trials if dictated by results from April and May trials
Sep - Jan 2011	Analyses of experimental data from laboratory trials
Nov 2011	<i>Progress report and presentation</i> to PFRP PIs Meeting (**)
Jan - Dec 2012	Modelling of ocean acidification impacts on Pacific Ocean yellowfin tuna by SEAPODYM (**)
August 2012	<i>Working Paper</i> to the Scientific Committee of WCPFC(IATTC??) reporting on the results from the laboratory trials
Nov 2012	<i>Report and presentation</i> to PFRP PI meeting (**)
Dec 2012	<i>Submission of papers to peer reviewed journals</i>
Aug 2013	<i>Working Paper</i> to the Scientific Committee of WCPFC(IATTC??) and discussion of the implications of ocean acidification for yellowfin tuna populations in the Pacific.

4.6 References

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Introduction

Dr Bromhead is currently a Senior Fisheries Scientist in the Stock Assessment and Modeling Section of the Oceanic Fisheries Programme at the Secretariat of the Pacific Community. Of relevance to this project, he has 8 years experience in the analysis and assessment of tuna fisheries data for the provision of scientific advice to fisheries managers at both national and WCPFC levels, in addition to 6 years experience in the assessment of the development, condition, growth and survival of early life history stages of teleosts (including Honours and PhD projects, as well as a first authored peer reviewed journal paper). He has significant experience in the use of the key analyses tools to be used in this project including generalised linear models (GLMs). The collaborators from SPC (Nicol, Hoyle), in addition to undertaking regional bycatch analyses and tuna stock assessments for the Western and Central Pacific Fisheries Commission, play a leading role in the provision of scientific advice from OFP to the WCPFC, have over 35 peer reviewed publications and also have extensive experience in the statistical analyses of experimental data such as will be collected under this project.

Education

Ph.D. (1997–2001): Australian National University (ANU). Thesis title: "Determining growth status in teleost larvae using flow cytometric cell cycle analysis"
Honours (1996): Australian National University (ANU). Thesis title: "The application of flow cytometric cell cycle analysis to the assessment of condition and growth in a freshwater fish *Galaxias olidus* (Gunther)".
Bachelor of Science (1992-1995): Australian National University (ANU) majoring in zoology, ecology, microbiology and biochemistry.

Relevant Employment History

Fisheries Scientist (2006-2008); Senior Fisheries Scientist (2008–Present)
Supervisors: Dr Shelton Harley (SheltonH@spc.int), Dr John Hampton (JohnH@spc.int)
Institution: Stock Assessment and Modelling Section Oceanic Fisheries Programme, Secretariat of the Pacific Community
Fisheries Scientist (2001–2006)
Supervisors: Dr James Findlay (James.Findlay@afma.gov.au), Dr John Kalish
Institution: Bureau of Rural Sciences Department of Agriculture, Fisheries and Forestry – Australia

Relevant Published papers

- Bromhead, D., Kalish, J. and Waring, P. (2000) Application of flow cytometric cell cycle analysis to the assessment of condition and growth in larvae of a freshwater teleost, *Galaxias olidus*. Canadian Journal of Fisheries and Aquatic Sciences. 57. 4. 732-741.
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Scientific Conference Papers and Funded Reports (selected)

- Bromhead, D., Hoyle, S., Williams, A., Wang, S., and Chang, S.K. (2009). Analysis of factors influencing the size of albacore tuna sampled from the South Pacific albacore longline fisheries. SA-IP-05. WCPFC Fifth Session of the Scientific Committee, Port Vila, Vanuatu, 10-21 August 2009.
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Relevant Books

- Gillett, R. and D. Bromhead (2009). Tuna for Tomorrow - Some of the Science Behind an Important Fishery in the Pacific Islands. Asian Development Bank and Secretariat of the Pacific Community, Manila, 50 pages.

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Education

Ph.D. 1986 University of Maryland
M.S. 1976 University of Massachusetts-Dartmouth
B.S. 1974 Michigan State University

Professional Experience

Senior Scientist, Inter-American Tropical Tuna Commission, La Jolla, CA (1988 to present)

Postdoctoral Fellow, National Marine Fisheries Service, La Jolla, CA (1986-1987)

Dean John A. Knauss Marine Policy Fellow, National Marine Pollution Program, NOAA, Rockville, MD (1985)

Graduate Research Assistant, Chesapeake Biological Laboratory, University of Maryland, Solomons, MD (1981-1985)

Associate Biologist, WAPORA, Inc., Environmental Consultants, Cincinnati, OH (1977-1980)

Recent Publications:

Margulies, D., M.C. Santiago, S.L. Hunt, J.M. Suter, S. Kimura, H. Nakata, J.B. Wexler, and V.P. Scholey. In review. The effect of microturbulence on the survival, feeding and growth of yellowfin tuna (*Thunnus albacares*) larvae. In Review for J. Plankton Research.

Margulies, D., J.M. Suter, S.L. Hunt, R.J. Olson, V.P. Scholey, J.B. Wexler, and A. Nakazawa. 2007. Spawning and early development of captive yellowfin tuna, *Thunnus albacares*. Fish. Bull. 105: 249-265.

Margulies, D., V.P. Scholey, J.B. Wexler, R.J. Olson, J.M. Suter, and S.L. Hunt. 2007. A review of IATTC research on the early life history and reproductive biology of scombrids conducted at the Ashotines Laboratory from 1985 to 2005. Inter-Am. Trop. Tuna Comm., Spec. Rep. 16. 63pp.

Wexler, J.B., S. Chow, T. Wakabayashi, K. Nohara, and D. Margulies. 2007. Temporal variation of *in situ* growth of yellowfin tuna (*Thunnus albacares*) larvae in the Panama Bight, 1990-1997. Fish. Bull. 105: 1-18.

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Wexler, J.B., V.P. Scholey, R.J. Olson, D. Margulies, A. Nakazawa, and J.M. Suter. 2003. Tank culture of yellowfin tuna, *Thunnus albacares*: developing a spawning population for research purposes. Aquaculture 220: 327-353.

Loew, E.R., W.N. McFarland, and D. Margulies. 2002. Developmental changes in the visual pigments of the yellowfin tuna, *Thunnus albacares*. Mar. Fresh. Behav. Physiol. 35: 235-246.

Margulies, D., J.B. Wexler, K.T. Bentler, J.M. Suter, S. Masuma, N. Tezuka, K. Teruya, M. Oka, M. Kanematsu, and H. Nikaido. 2001. Food selection of yellowfin tuna, *Thunnus albacares*, larvae reared in the laboratory. Inter-Am. Trop. Tuna Comm., Bull. 22: 9-51.

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Margulies, D. 1997. Development of the visual system and inferred performance capabilities of larval and early juvenile scombrids. Mar. Fresh. Behav. Physiol. 30: 75-98.

Professional Affiliations

American Fisheries Society (AFS): Early Life History Section (Western Regional Representative)

AFS Marine Fisheries Section, Fish Culture Section, and International Section

CLIOTOP Program (Climate Impacts on Oceanic Top Predators), IMBER Programme (Integrated Marine Biogeochemistry and Ecosystem Research), Steering Committee Member, Working Group 1: Early Life History of Top Predators

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CURRENT POSITION and RESEARCH ACTIVITIES

LABORATORY DIRECTOR, Inter-American Tropical Tuna Commission (IATTC), Achotines Laboratory, Rep. of Panama, 1985-present.

Supervise 20-member international staff at only facility in the world with yellowfin tuna spawning in captivity since 1996. Resulting eggs and larvae used for research on optimal methods and conditions for culture of larval and juvenile scombrids. Ongoing field and lab research on tuna early life history, biology, and ecology.

AREAS OF EXPERIENCE

- Collection, analysis, and presentation of technical and scientific information
- Supervision and management of Human Resources
- Supervise research-scale hatchery operation for marine fish
- Capture, transfer, and maintenance of pelagic fish for use as broodstock

TECHNICAL EXPERTISE

- Design and construction of seawater/culture systems
- Use of microcomputers; word-processing, spreadsheet, database, and statistics
- SCUBA basic and advanced certification, U/W photography
- Aircraft owner and pilot

EDUCATION

- M.Sc. in Fisheries, College of Ocean & Fishery Sciences, University of Washington, 1993
- B.Sc. in Fisheries, School of Fisheries, University of Washington, 1975

LANGUAGES

- Fluent in Spanish, Japanese and German spoken well

HONORS AND AWARDS

- Appointed by the President of Panama, Martín Torrijos Espino, on April 13, 2007, as one of five members of the Board of Directors of the Secretaría Nacional de Ciencia, Tecnología e Innovación (SENACYT) of Panama.

RECENT CONFERENCES ATTENDED

- 1st International Mariculture Conference, Manta, Ecuador. April 15-16, 2010. Invited Speaker
- Graduate lecture program at Kinki University, Nara Prefecture, Japan, November 25-27, 2008, Presented summary of tuna research program at Achotines Laboratory. Invited speaker.
- Workshop “Developing a Sustainable Aquaculture Industry in the Azores.” Horta, Faial (Azores). June 2-5, 2008. Invited Keynote Speaker.
- Seminar to Coordinate Fisheries Resource Research Methodology. Oct 8-10, 2007. Antigua, Guatemala. Invited Keynote Speaker.

- 3rd Colombian Aquaculture Congress. Santa Marta, Colombia. October 4-6, 2006. Invited Keynote Speaker. Following Congress taught an intensive one-week graduate level course on marine fish culture at the Universidad de Magdalena.
- International Seminar on Tuna Fisheries. Denpasar, Bali, Indonesia. May 14-15, 2005. Invited Keynote Speaker.

PUBLICATIONS

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- Margulies, D., J.M. Suter, S.L. Hunt, R.J. Olson, V.P. Scholey, J.B. Wexler, and A. Nakazawa. 2007. Spawning and early development of captive yellowfin tuna, *Thunnus albacares*. Fish. Bull. 105: 249-265.
- Margulies, D., V.P. Scholey, J.B. Wexler, R.J. Olson, J.M. Suter, and S.L. Hunt. 2007. A review of IATTC research on the early life history and reproductive biology of scombrids conducted at the Achotines Laboratory from 1985 to 2005. IATTC Spec. Report 16: 63 pp.
- Takagi, M., Chow, S., Okamura, T., Scholey, V., Nakazawa, A., Margulies, D., Wexler, J., Taniguchi, N., 2003. Mendelian inheritance and variation of four microsatellite DNA markers in the yellowfin tuna *Thunnus albacares*. Fish. Sci., 69 (6): 1306-1308.
- Wexler, J. B., Scholey, V.P, Olson, R. J., Margulies, D., Nakazawa, A., Suter J. M., 2003. A land-based culture system for yellowfin tuna, *Thunnus albacares*: developing a spawning population for research purposes. Aquaculture 220: 327-353.
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- Olson, R.J., and V.P. Scholey. 1990. Captive Tunas in a Tropical Marine Research Laboratory: Growth of Late-larval and Early-juvenile Black Skipjack *Euthynnus lineatus*. Fishery Bulletin, U.S. 88:821-828.

5. Budget

YEAR 1

Item	Year 1 (USD)	Comments
Personnel	60,000	IATTC laboratory technician salary support (30000); Salary and travel to Achatines for IATTC scientists (20000); Chemical oceanographer salary to advise on water chemistry and appropriate treatment levels in lab trials (10000)
Equipment purchase	32,000	Additional tanks, filters, CO2 regulation and other laboratory equipment
Equipment freight/duty	3,000	Costs associated with bringing equipment into Panama and to Achatines
Travel	20,000	Travel by SPC staff to Achatines for the trials, and to PFRP PI meetings
Subtotal	115,000	
SPC Indirect cost rate	15%	
SPC Indirect costs	17,250	
UH Indirect cost rate	5,150	20.6% of the first 25k (USD5,150)
TOTAL	137,400	

YEAR 2

Item	Year 2 (USD)	Comments
Personnel	30,000	CLS technical staff salary support (20000). Salary for University of Hawaii geochemical oceanographic modeller to provide predicted physical oceanography data layers for SEAPODYM and associated advice on use of that data (10000)
Travel	15,000	Travel by PIs to PFRP PI workshop
Subtotal	45,000	
SPC Indirect cost rate	15%	
SPC Indirect costs	6,750	
TOTAL	51,750	

Note:

1. The addition of USD 10 000 for a chemical oceanographer in year 1, above the budget stated in the pre-proposal, is a response to the recommendation from the PFRP (stated in the pre-proposal acceptance letter) that water chemistry elements of the trials would need very careful consideration to ensure that they mimicked chemical changes occurring under carbon induced ocean acidification, and that treatment levels chosen were relevant to tuna habitat. An initial response from Dodie Lau indicated that this inclusion appeared reasonable from an administrative and financial viewpoint.
2. The original pre-proposal only included the SPC Indirect cost and not the UH Indirect cost which the pre-proposal acceptance letter indicated we must include.

6. Prior PFRP awards

None of the principle investigators on this proposal have received prior PFRP awards.

7. Accomplishments arising from prior PFRP awards

Not applicable

8. Current and pending support

The **IATTC ELH group and Achotines Laboratory** currently have support for the following four projects:

1. Project Title: Study of methods to collect, transfer, and culture indo-pacific sailfish and wahoo at Achotines Laboratory, Republic of Panama; Date: May 1, 2007 through June 30, 2011
Funding Institution: [Nacional Secretary of Science, Technology, and Innovation](#)
Amount of Award: US\$100,000.00 over life of project
Person-months devoted by PI: An average of 1.5 months/year by Vernon Scholey; an average of 0.5 months/year by Daniel Margulies
2. Project Title: Develop techniques to culture, maintain, and optimize marine microalgae with the goal of organizing a collection of species used in aquaculture
Date: May 1, 2007 through June 30, 2010
Funding Institution: [Nacional Secretary of Science, Technology, and Innovation](#)
Amount of Award: US\$50,000.00 over life of project
Person-months devoted by PI: An average of 0.25 months/year by Vernon Scholey
3. Project Title Advancement of Hatchery Technologies for Commercial-Scale Production of California Yellowtail (*Seriola lalandi*) and Yellowfin Tuna (*Thunnus albacares*)
Date: : July 1, 2009 through June 30, 2011
Funding Institution: Saltonstall-Kennedy Program of the U.S. National Oceanic and Atmospheric Administration

Amount of Award: US\$41,500.00 per year

Person-months devoted by PI and Collaborating Scientists: An average of 1.5 months/year by Vernon Scholey; an average of 2.0 months/year each by Daniel Margulies, Jeanne Wexler, and Maria Santiago

4. Project Title Comparative Studies of the Early Life History of Pacific Bluefin Tuna (*Thunnus orientalis*) and Yellowfin Tuna (*Thunnus albacares*) for Purposes of Resource Management and Aquaculture Development

Date: April 1, 2010 through March 31, 2015

Funding Institution: Japan Science and Technology Agency (JST)

Amount of Award: Final funding amounts are pending

Person-months devoted by PI and Collaborating Scientists: Estimated at an average of 2.0 months/year by Vernon Scholey, 4.0 months/year by Daniel Margulies, 3.0 months/year for Jeanne Wexler, and 6.0 months/year by Maria Santiago

Dr Don Bromhead (**Oceanic Fisheries Programme of SPC**) is fully funded by core Programme (SPC) funding (which totals USD 1 million) and salary is not linked to any particular project or institution.

9. Contact details for potential reviewers

Dr. Richard Brill

Virginia CMER Program Director
Virginia Institute of Marine Science
Gloucester Point, VA 23062 USA
Phone (804) 684-7773
FAX (808) 592-8300
email: rbrill@vims.edu

Daniel Benetti

Chairman, Division of Marine Affairs & Policy;
Associate Professor and Director of Aquaculture,
RSMAS, University of Miami
Telephone: +1 305-421-4889
Fax: +1 305-421-4675
Email: dbenetti@rsmas.miami.edu

Dr James C. Orr (james.orr@lsce.ipsl.fr)
Commissariat à l'Énergie Atomique (CEA)
Laboratoire des Sciences du Climat et de l'Environnement,
CEA Saclay, Gif-sur-Yvette Cedex, France.

Dr Ken Caldeira (kcaldeira@dge.stanford.edu)
Carnegie Institution Department of Global Ecology,
Stanford, CA, USA.

Prof. Victoria Fabry (fabry@csusm.edu)

Department of Biological Sciences, California State University at San Marcos,
San Marcos, CA, USA.

Dr Jean-Pierre Gattuso (gattuso@obs-vlfr.fr)

Centre National de la Recherche Scientifique (CNRS)

Laboratoire d'Océanographie, CNRS and Université Pierre et Marie Curie,
Villefranche-sur-mer, France.

**ATTACHMENT 1 – LETTERS OF COMMITMENT FROM THE KEY INSTITUTIONS,
PRINCIPLE INVESTIGATORS AND COLLABORATING SCIENTISTS**

**SECRETARIAT OF THE
PACIFIC COMMUNITY**

BPD5
98848, Noumea Cedex
New Caledonia

TELEPHONE: +687 26.20.00
FAX: +687 26.38.18
E-mail: spc@spc.int



**SECRETARIAT GÉNÉRAL DE LA
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Mél : spc@spc.int

14 May 2010

Dr. Kevin C. Weng
PFRP Program Manager
Pelagic Fisheries Research Program
1000 Pope Road
Marine Science Bldg. 312
Honolulu, HI 96822

Dear Dr Weng,

I would like to advise you of the Secretariat of the Pacific Community's support for the PFRP proposal "Ocean Acidification Impacts Upon Tropical Tuna Populations" by Bromhead, Margulies, and Scholey. The project is the first step towards ensuring that RFMOs and their members can incorporate knowledge of ocean acidification impacts upon target tuna populations into management decisions aimed at achieving their key sustainability objectives. The Oceanic Fisheries Programme of SPC is committed to providing the support necessary to make the project successful.

Yours sincerely

Dr John HAMPTON
Oceanic Fisheries Programme
Secretariat of the Pacific Community

COMISION INTERAMERICANA DEL ATUN TROPICAL INTER-AMERICAN TROPICAL TUNA COMMISSION

8604 La Jolla Shores Drive, La Jolla CA 92037-1508, USA – www.iattc.org
Tel: (858) 546-7100 – Fax: (858) 546-7133 – Director: Guillermo Compeán

13 May 2010
Ref: 0229 -860

Dr. Kevin C. Weng
Interim Program Manager
Pelagic Fisheries Research Program
University of Hawaii at Manoa
Joint Institute for Research and Atmospheric Research
1000 Pope Road, MSB 312
Honolulu, Hawaii 96822

Dear Dr. Weng,

I would like to advise you of the Inter-American Tropical Tuna Commission's support for the PFRP proposal "Ocean acidification impacts upon tropical tuna populations" by Bromhead, Margulies, and Scholey. The project objectives directly relate to the improvement of tuna stock assessments in the Pacific Ocean and we are committed to supporting the project. The IATTC will provide laboratory facilities at the Achotines Laboratory to conduct the proposed experimental work, and IATTC scientists will collaborate with the project scientists to analyze the experimental data collected during the study.

Yours sincerely,



for
Guillermo Compeán
Director

**SECRETARIAT OF THE
PACIFIC COMMUNITY**

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14 May 2010

Dr Don Bromhead
Senior Fisheries Scientist
Oceanic Fisheries Programme
Secretariat of the Pacific Community
BP D5
Noumea CEDEX 98848
New Caledonia

Dr. Kevin C. Weng
PFRP Program Manager
Pelagic Fisheries Research Program
1000 Pope Road
Marine Science Bldg. 312
Honolulu, HI 96822

Dear Dr Kevin Weng,

Thank you for inviting us to submit a full version of the proposal entitled "*Ocean acidification impacts upon tropical tuna populations*" to the PFRP. As a PI on the proposed project, I am writing to indicate my commitment to the project and to represent the work at PFRP events and activities.

Sincerely

Don Bromhead

COMISION INTERAMERICANA DEL ATUN TROPICAL INTER-AMERICAN TROPICAL TUNA COMMISSION

8604 La Jolla Shores Drive, La Jolla CA 92037-1508, USA – www.iattc.org
Tel: (858) 546-7100 – Fax: (858) 546-7133 – Director: Guillermo Compeán

Date: May 11, 2010

Ref: 0217-860

Pelagic Fisheries Research Program
University of Hawaii at Manoa
Joint Institute for Research and Atmospheric Research
1000 Pope Road, MSB 312
Honolulu, Hawaii 96822

To Whom It May Concern:

I am writing to express my commitment to participate as a Co-Principal Investigator in the project entitled, “Ocean Acidification Impacts Upon Tropical Tuna Populations.” This proposal is being submitted to the Pelagic Fisheries Research Program through colleagues at the Secretariat of the Pacific Community (SPC).

This proposal addresses a topic of increasing importance in global marine sciences – the potential effects of ocean acidification on marine ecosystems. The IATTC’s Achatines Laboratory is uniquely qualified to support experimental studies of the effects of ocean acidification on early life stages of tropical tunas, and I look forward to participating in this project.

Sincerely,



Daniel Margulies
Senior Scientist

INTER-AMERICAN TROPICAL TUNA COMMISSION
COMISION INTERAMERICANA DEL ATUN TROPICAL
ACHOTINES LABORATORY
LABORATORIO ACHOTINES

Las Tablas, Provincia Los Santos
Republica de Panama
Tel: (507) 995-8166
Fax: (507) 995-8282

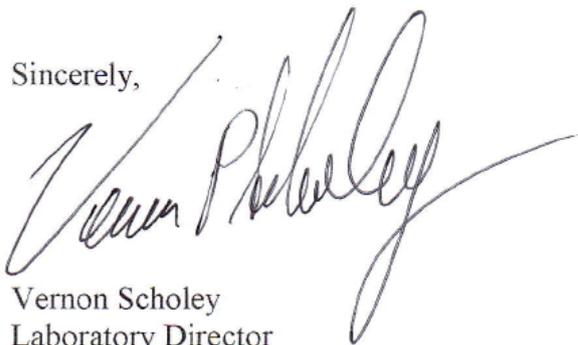
April 28, 2010

Pelagic Fisheries Research Program
University of Hawaii at Manoa
Joint Institute for Research and Atmospheric Research
1000 Pope Road, MSB 312
Honolulu, Hawaii 96822

Greetings:

I am writing to confirm my willingness to participate in the "Ocean Acidification Impacts on Tropical Tuna" project and also to represent the work at PFRP events and activities.

Sincerely,

A handwritten signature in black ink, appearing to read "Vernon Scholey". The signature is fluid and cursive, with a long horizontal stroke extending to the right.

Vernon Scholey
Laboratory Director

**SECRETARIAT OF THE
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Dr Simon Nicol
Principal Fisheries Scientist
Oceanic Fisheries Programme
Secretariat of the Pacific Community
BP D5, 98848 Noumea CEDEX,
New Caledonia

Dr. Kevin C. Weng
PFRP Interim Program Manager
Pelagic Fisheries Research Program
1000 Pope Road
Marine Science Bldg. 312
Honolulu, HI 96822

13 May 2010

Dear Dr Kevin Weng,

Thank you for inviting us to submit a full version of the proposal entitled "*Ocean acidification impacts upon tropical tuna populations*" to the PFRP. I am named as a collaborator on the proposed work, and I am sending you this letter to indicate my willingness to participate in all aspects of the project. I will participate in the meetings of the project and will contribute to the writing of the reports and scientific paper(s). If you have any questions regarding my willingness to participate in the proposed research or my role, please contact me.

Sincerely Yours,

Simon Nicol

**SECRETARIAT OF THE
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13 May 2010

Dr Simon Hoyle
Senior Fisheries Scientist
Oceanic Fisheries Programme
Secretariat of the Pacific Community
BP D5
Noumea CEDEX 98848
New Caledonia

Dr. Kevin C. Weng
PFRP Program Manager
Pelagic Fisheries Research Program
1000 Pope Road
Marine Science Bldg. 312
Honolulu, HI 96822

Dear Dr Weng,

Thank you for inviting us to submit a full version of the proposal entitled "*Ocean acidification impacts upon tropical tuna populations*" to the PFRP. As a collaborator on the proposed work, I am writing to indicate my commitment to the project.

Sincerely,

Simon Hoyle

COMISION INTERAMERICANA DEL ATUN TROPICAL
INTER-AMERICAN TROPICAL TUNA COMMISSION

8604 La Jolla Shores Drive, La Jolla CA 92037-1508, USA – www.iattc.org
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May 11, 2010
Ref: 0214-860

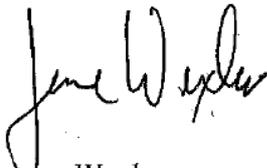
Pelagic Fisheries Research Program
University of Hawaii at Manoa
Joint Institute for Research and Atmospheric Research
1000 Pope Road, MSB 312
Honolulu, Hawaii 96822

To Whom It May Concern:

This letter serves to express my commitment to participate as a Collaborating Scientist in the project entitled, “Ocean Acidification Impacts Upon Tropical Tuna Populations.” This proposal is being submitted to the Pelagic Fisheries Research Program through colleagues at the Secretariat of the Pacific Community (SPC).

This proposal addresses the potential effect of ocean acidification on marine ecosystems and is an important topic in understanding the effects of changing global ocean conditions on tuna populations. The IATTC’s Achotines Laboratory is uniquely qualified to support experimental studies of the effects of ocean acidification on early life stages of tropical tunas, and I look forward to participating in this project.

Sincerely,



Jeanne Wexler
Associate Scientist

COMISION INTERAMERICANA DEL ATUN TROPICAL INTER-AMERICAN TROPICAL TUNA COMMISSION

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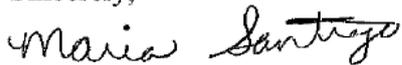
Date: May 11, 2010
Ref: 215-860

Pelagic Fisheries Research Program
University of Hawaii at Manoa
Joint Institute for Research and Atmospheric Research
1000 Pope Road, MSB 312
Honolulu, Hawaii 96822

To Whom It May Concern:

Please accept this as my letter of commitment to participate as a collaborator in the project entitled, “Ocean Acidification Impacts Upon Tropical Tuna Populations,” should it be accepted for funding. I look forward to being involved in this important research.

Sincerely,



Maria Santiago
Assistant Scientist



Dr Patrick Lehodey
Chief, Marine Ecosystem Modeling
Space Oceanography Division
CLS, 8-10 rue Hermes
31520 Ramonville
France

Dr. Kevin C. Weng
PFRP Interim Program Manager
Pelagic Fisheries Research Program
1000 Pope Road
Marine Science Bldg. 312
Honolulu, HI 96822

12 May 2010

Dear Dr Kevin Weng,

Thank you for inviting us to submit a full version of the proposal entitled "*Ocean acidification impacts upon tropical tuna populations*" to the PFRP. I am named as a collaborator on the proposed work, and I am sending you this letter to indicate my willingness to participate in the work and the role that I will play in it.

My colleagues and I have agreed that I would be in charge during the second year of the project of adapting the SEAPODYM model to include impacts of changing pH on the larvae mortality based on the results of laboratory experiments, and then to run a simulation for yellowfin under a climate change scenario. I will participate to the meetings of the project and will contribute to the writing of the reports and scientific paper(s).

If you have any questions regarding my willingness to participate in the proposed research or my role, please contact me.

Sincerely Yours,

Patrick Lehodey