Interacting Effects of Climate Change Variables on Fish Aerobic Metabolism: Changing Exercise Performance in the Face of Deoxygenation, Hypercapnia, and Hyperthermia

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1. Introduction

1.1. Abstract

A large body of recent research has attempted to elucidate and forecast the impacts of climate change on marine teleosts. Although there have been many important contributions to our understanding, two key areas that have received relatively little attention are interactions between multiple stressors, and the development of a mechanistic, rather than purely statistical predictive framework. This thesis attempts to address these gaps by examining the effects of multi-stressor climate change perturbations on the capacity for aerobic metabolism in two marine teleosts – the yellowfin tuna (*Thunnus albacares*) and the Mozambique tilapia (*Oreochromis mossambicus*). The methods used in the investigation will range from theory-based physiological models (Chapter I), direct experimentation on captive animals (Chapter II) and empirical data-based forecasts of future performance (Chapter III). Put together, these chapters demonstrate a variety of complementary, physiology-based approaches to understanding and predicting the response of marine teleosts to complex environmental change. This introduction will seek to provide some background on the energetic terms used—particularly the concept, and methods of measurement for the aerobic scope—as well as considerations of different approaches to understanding population responses to future change.

1.2. Teleost Ecology in a Climate Change Future

The oceans are expected to undergo substantial changes due to anthropogenic climate change in the near future. Estimates indicate that end-century global sea-surface warming is likely to reach close to 3°C under a RCP 8.5 greenhouse gas emissions scenario (also known as the “business as usual” scenario), while sea surface pH is likely to fall by 0.35 pH units and global ocean oxygen content is likely to decline by close to 4% (Bopp et al., 2013). These changes are driven by atmospheric warming, increased dissolved inorganic carbon (DIC) due to the diffusion of anthropogenic CO_2 into the oceans (driving a decline in ocean pH), and by surface ocean stratification lowering the rate of transport of oxygenated, mixed-layer water into the oxygen minimum layer (Gruber, 2011). Furthermore, there is concern that strong interactions between these drivers could amplify future perturbations beyond what is predicted by the ensemble average of global climate models (Gruber, 2011). One such interaction that is not captured in many models is the potential impact of ocean pH on the speed and effectiveness of the biological carbon pump (Hoffman and Schellnhuber, 2009). Low pH (and the resultant decline in carbonate saturation) may reduce the mass of the mineral skeletons of many planktonic organisms by inhibiting biological calcification. The resulting slow-down in the rate of sinking of dead calcifying plankton would cause more decomposition to occur at shallower depths, intensifying the oxygen minimum layer and raising CO_2 concentrations at these depths due to the stoichiometry of the Redfield Ratio (Brewer and Peltzer, 2009).

Regardless of these uncertainties in the magnitude of these changes, it is clear that there will be profound impacts on marine biogeochemistry and ecosystem functioning (Gruber, 2011). While much research has focused on the potential impacts on calcifying organisms such as corals and pteropods (Hoegh-Guldberg et al., 2007), there is strong uncertainty as to how teleosts, which account for over half of the world’s vertebrate species and 6% of the total protein intake for the human population (Allison et al., 2009), will be affected (Le Quesne and Pinnegar, 2011). Studies have variously indicated strongly negative, positive, or no impacts of climate-related variables on teleosts, sometimes even within the same species (Cruz-Neto and Steffensen, 1997; Mckenzie et al., 2003; Melzner et al., 2009; Moran and Støttrup, 2011).

Two key issues that have received comparatively little attention in this field are the effect of interactions between multiple climate change variables on determining biological outcomes, and the development of a mechanistic framework for understanding and predicting climate change effects. The first issue is of critical importance because climate change in the marine environment is fundamentally a multi-stressor phenomenon, with changes in temperature, oxygen, and pCO_2 expected to co-occur across large regions of the oceans (Bopp et al., 2013). Furthermore, recent evidence suggests that simultaneous stressors rarely interact additively (Crain et al., 2008), thereby precluding the assumption that studies on organism responses to single stressors can be accurately applied to the multiple stressor case. Although this issue has long been identified as a key challenge across many fields of ecology and conservation biology (Breitburg et al., 1999; Crain et al., 2008), the complexity of the experiments necessary to investigate it have discouraged more scrutiny. Nevertheless, at a minimum the interactions between the three core variables of temperature, oxygen, and pCO_2 need to be better understood if we are to understand the potential impacts of climate change more fully.

The second issue of the need for a more mechanistic model is also a fundamental one. This is because climate change prediction is almost always an extrapolative exercise in that forecasts must deal with species’ responses to future temperature, oxygen, or pCO_2 conditions that are not observed in their current habitats (Kearney and Porter, 2009). The most common methodology of predicting the impacts of climate change involve some form of statistical correlation between current species distributions and observed environmental variables (Denny and Helmuth, 2009). Such correlations are singularly unsuited to extrapolation and thus must be treated with care in the climate change context (Kearney and Porter, 2009). Recently, the development of the aerobic scope concept as a coherent mechanistic framework for understanding the impacts of environmental change on marine species has become a promising avenue for moving beyond such correlative models (Pörtner, 2010). Unfortunately this approach is currently not widely used, and to our knowledge has been limited to simple single-stressor models that only predict presence or absence in a given future habitat.

1.3. Understanding Aerobic Scope

The concept of a scope for aerobic metabolism was first suggested by Fry (1947) as an organism-level indicator of the effect of environmental factors on animal activity. Since then, it has been used by investigators to study the effects of temperature, oxygen, salinity, pCO_2, disease burden, and a host of other factors on a variety of species ranging from mammals to teleosts (Khan et al., 2014; Killen et al., 2007; Lapointe et al., 2014; Nilsson et al., 2009; Pörtner, 2002; Weibel et al., 2004). More broadly speaking, the concept that an energy budget, including not just aerobic scope but scope for growth (Stumpp et al., 2011; Widdows et al., 1997), and mass-balance bioenergetics (Brandt et al., 2009; Kearney and Porter, 2009) influences population viability and distribution has found wide acceptance across many fields of ecology and conservation biology.

The key insight elucidated by Fry is that the capacity of an organism for aerobic metabolism can be partitioned into two parts. The first is the minimum metabolic expenditure necessary to sustain life, and this mandatory metabolic output is commonly termed the standard metabolism (in analogy to the basal metabolism, which describes the minimum metabolic rate in endotherms under the special case where there is no metabolic cost of thermoregulation) (Hochachka et al., 2003). This standard metabolic rate is typically defined as including such activities as maintaining a constant osmolality of internal fluids, a minimum survivable level of ventilation and gas exchange, and maintenance of existing tissues. Activities that are excluded from the standard metabolic rate include growth (both somatic and gonadal), digestion, and locomotion. These optional activities contribute to the active metabolic rate, which is the maximum aerobic metabolism that can be sustained under maximum exercise (e.g. fast swimming) or a combination of exercise and digestion (Alsop and Wood, 1997). By subtracting the standard metabolic rate from the active metabolism, the remaining quantity, termed the aerobic scope, describes the total energy budget available for non-life-sustaining processes.

Due to years of study, a number of frameworks have been developed to describe the changes in aerobic scope in response to a gradient of environmental conditions. The most prominent of these is the oxygen-limited thermal tolerance hypothesis (Pörtner, 2001), which states that the power-law scaling of standard metabolic rates with temperature compared with the bell-shaped relationship for active metabolic rates necessarily results in a temperature optimum (T_{opt}) for aerobic scope and an upper and lower critical temperature (T_{crit}) at which aerobic scope falls to zero (Figure 1a). Within a few degrees of the optimum temperature there is typically a thermal tolerance zone where changes in temperature have little effect on the aerobic scope. The boundaries of this temperature range are described by the upper and lower *pejus* temperatures (T_{pejus}) beyond which further thermal stress causes a rapid decline in aerobic scope (Pörtner et al., 2001). These parameters, T_{opt},

T_{crit}, and T_{pejus} can be used to describe the impact of a range of ambient temperatures on aerobic fitness under a given set of environmental conditions (Farrell et al., 2009). The addition of simultaneous stressors impacting the oxygen uptake or demand of an organism can alter the thermal tolerance by changing the capacity for a high active metabolism Such stressors include low oxygen (hypoxia) or high pCO_2 (hypercapnia), both of which are thought to decrease active metabolism leading to a narrowing of the *pejus* and critical temperature limits (Figure 1b).

A second framework that has been used to understand changes in aerobic scope is the limiting oxygen concentration continuum (Claireaux and Lagardére, 1999), which states that any given metabolic rate has a limiting oxygen concentration below which that metabolic activity cannot be sustained. As a result, active metabolic rates are observed to rise monotonically with oxygen concentration, reaching an asymptote at a point very close to air saturation in fully active individuals (Figure 2). Under this framework, simultaneous stressors such as high or low temperature (hypo- or hyperthermia) or hypercapnia leads to a decline in the maximum active metabolic rate (the active metabolism under air saturation) and a narrowing of the critical limits (Claireaux et al., 2000).

1.4. Metabolic Linkages Between Aerobic Scope and Fitness

The basis for the focus on aerobic scope as a predictor of environmental impacts on organism physiology is the fact that it describes a fundamental and mechanistic constraint on population abundance and distribution. More specifically, the aerobic scope can be thought to define a “fundamental niche” (Hutchinson, 1957), which is to say that it describes the impact of abiotic factors, but not the biotic interactions that determine the “realized” or observed niche (Kearney and Porter, 2009). This is because the activities that drive organismal fitness – growth, reproduction, foraging, digestion, and locomotion – are all fueled by the non-maintenance aerobic metabolism that makes up the aerobic scope (Pörtner et al., 2001) either directly in the case of a purely aerobic activity, or indirectly, through the excess post-exercise oxygen consumption (EPOC) that is required to compensate for anaerobic expenditures (Gaesser and Brooks, 1983). In the absence of biotic change (e.g. changes in forage availability) declines in aerobic scope due to adverse environmental conditions necessarily reduce the capacity for one or more of these activities (Farrell et al., 2009). This is reflected in the multiple studies that have found close and significant correlations between aerobic scope and growth, appetite, or locomotory ability (Table 1).

In essence, the aerobic scope describes an integrative bioenergetics budget for the activities most closely tied to fitness. This is a key difference when compared against other fitness indicators such as somatic growth rate, which may be conserved in organisms faced with environmental stressors at the expense of other fitness-related activities (e.g. gonadal growth or immune functioning) or modulated to maintain stable levels of these activities (Lambert and Dutil, 2000; Rijnsdorp, 1990). As a result, such indicators may exaggerate or fail to detect the impact of an environmental stressor on an organism even under conditions that elicit a marked decline in aerobic scope. Furthermore, predictions of future growth rate in such studies rely upon the implicit assumption that the proportion of available aerobic energy that is allocated to growth will remain stationary under future conditions. Transient bionomic factors such as the short-term modulation of endogenous hormone levels (e.g. the GH/IGF axis), which are difficult to predict with any certainty, can strongly influence the allocation of energy to different activities (Schreck, 2010). The ability to relax this assumption is especially important for applications such as climate change prediction, where changes in population or ecosystem dynamics could easily influence these factors on a population-scale.

1.5. Methods of Aerobic Scope Measurement

Aerobic scope measurements require two components: (1) a method of measuring the aerobic metabolic rate of the study animal, and (2) a method of modulating its activity. While manual exhaustion (chasing of fishes by investigators with hands/sticks) and feeding-based metabolic activity has been used previously (Lefrançois and Claireaux, 2003; Vanella et al., 2010), the most popular and standardized methodology for aerobic scope measurement is the swim tunnel respirometer. This combines a sealed chamber for measuring the oxygen uptake resulting from an animal's metabolism with a recirculating system that encourages the fish to swim at the desired speed. Because most teleosts exhibit a rheotactic response, raising the speed of recirculation through a respirometer chamber forces the fish to match its swimming speed to the current to maintain its position (Dewar and Graham, 1994). At swimming speeds above the critical swimming speed (U_{crit}) defined as the highest swimming speed that can be maintained over prolonged periods (Plaut, 2001), a fish must rely on some combination of maximal aerobic metabolism and supplemental anaerobic metabolism to meet the energetic demands of locomotion. The active metabolic rate measured during this speed of swimming can be compared against the metabolic rate achieved during prolonged quiescence in a respirometer chamber with little or no flow, typically with the subject resting against the bottom of the chamber. In the case of small study animals, the chamber is often designed as a

Blazka-type respirometer (Blazka, 1960) with internal recirculation, whereas for larger fishes a Brett-type design (Brett, 1964) with the recirculation flow routed through a system of pipes is often used.

Respirometer designs can be further subdivided into continuous-flow or intermittent-flow systems based on whether fresh oxygenated water is allowed to flow continuously into the swim chamber, thereby maintaining a constant internal dissolved oxygen concentration, or only intermittently, typically every ten to fifteen minutes. In the continuous flow case, the metabolic oxygen consumption must be measured based on the differential in dissolved oxygen concentration between points upstream and downstream of the fish, and the measurements depend upon strict assumptions of the flow regime within the swim chamber. In the intermittent flow case, the decline of dissolved oxygen over time during the period when the chamber is sealed (i.e. no new oxygenated water is allowed to flow in) is used to measure the metabolic oxygen consumption. While this results in a small oscillation in the oxygen concentration within the chamber and limits the time-resolution of metabolic measurements, this method is commonly accepted to be a more reliable mode of metabolic measurement (Steffensen, 1989).

Our aerobic scope measurements rely upon a custom-designed Blazka-type intermittent flow respirometer diagrammed in Figure 3. Our custom-built swim-tunnel respirometer is unique to our knowledge due to its ability to simultaneous modulate not only the oxygen concentration within the swim chamber but also the pCO_2 and temperature, allowing us to investigate the impacts of multi-stressor perturbations involving all of these variables in an experimental setting.

1.6. Predicting Aerobic Scope: A Diversity of Approaches

The approaches for predicting the future aerobic scope of a species or population reside within the wide spectrum of species distribution and abundance models that currently exist. For example, the most heavily statistically driven models of species distribution rely on correlations between presence/absence data and observed environmental characteristics. These are called climate envelope, habitat suitability, or niche models, and are a powerful and generalizable method of predicting species responses to climate change and other complex environmental challenges (Hijmans and Graham, 2006; Hirzel et al., 2006; Thuiller, 2004). More mechanistic or experimental approaches include bioenergetics modeling, biophysical models, and the use of experimental data on environmental tolerance to construct estimates of a niche space (Homyack, 2010; Kearney and Porter, 2009). While these models suffer from the difficulties of scaling organism physiology to population-level changes and are less likely to fit the data used for evaluation, they offer the advantages of being more easily ecologically interpretable and also of being more robust to non-equilibrium conditions and extrapolation to novel environments (Denny and Helmuth, 2009). In addition, they are less likely to conflate explanatory variables by spuriously attributing a limit in species range imposed by the latitudinal distribution of its prey for example on a temperature limitation (Homyack, 2010). Many of these advantages and disadvantages are summarized in Table 2.

Current approaches to predicting environmental impacts on populations using aerobic scope have focused on direct interpolation/extrapolation of experimental results. This type of forecasting has been used repeatedly for example to predict the response of migrating salmon to increases in the water temperature of their native streams (Eliason et al., 2011; Farrell et al., 2009, 2008). Such models typically measure variation in aerobic scope against a single environmental variable (most often temperature), interpolate a simple statistical function that outputs aerobic scope within a range of that environmental variable, and then predict future aerobic scope under different scenarios of unidimensional environmental change. This is despite the fact that the techniques for measuring the impact of multiple variables on aerobic scope have been demonstrated multiple times (Claireaux and Lagardére, 1999; Claireaux et al., 2000; Lapointe et al., 2014; Munday et al., 2009), and despite repeated efforts in the niche modeling community to point out the overwhelming importance of multiple-stressor challenges when predicting responses to large-scale environmental shifts such as climate change (Bopp et al., 2013; Crain et al., 2008). This thesis attempts to extend this methodology to more sophisticated environmental change scenarios, using three methods: (1) a novel theoretical aerobic scope model based on measured physiological traits (Chapter I), (2) a set of empirical measurements of aerobic scope on captive animals in response to multidimensional environmental change (Chapter II), and (3) an empirical model informed by the oxygen-limited thermal tolerance and limiting oxygen continuum frameworks described above to interpolate between the measurements obtained in Chapter II (Chapter III). Collectively, these three approaches share many of the advantages described in Table 2. We will use the theoretical model developed in Chapter I for example to directly assess the capacity for adaptation to novel environmental conditions due to physiological plasticity/selection of already extant traits, and we will evaluate this model against independent data on species occurrence. We will use the experimental results obtained in Chapter II to examine the possible impacts of a range of novel environments on aerobic fitness. Finally, we will use the empirical model developed in Chapter III to interpret the possible consequences of changes in aerobic scope on organismal physiology.

1.7. Acknowledgements

In order to test the questions posed in this thesis, a variety of approaches encompassing a large knowledge base were required. Chapter I required a deep dive into the world of physiological modeling as well as the ability to parse a complex CMIP5 dataset, while Chapter II led away from the computer screen and into the machine shop, where a great deal of engineering was required to develop the custom designed and built respirometry system. Because triple stressor experiments require more controls than traditional respirometry, I ended up writing my own software to control the system. Finally, Chapter III required the programming expertise to analyze large amounts of data. I would not have been able to do these things without the guidance of my advisor and my committee who helped to develop this thesis, especially Kevin's knowledge of tuna biology and the tuna-research community, Andre's expertise on tilapia, David's guidance on gas control and measurement, Anna's help on combing the literature, and Jeff's advice on electronics and especially on my early (aborted) ideas of an oxygen-sensing buoy. I would also like to thank my friends, especially my roommate Michelle, and my fiancé Xue who helped me to get over the many frustrations of coding and especially of plumbing a respirometer system.

1.8. List of References

Allison, E.H., Perry, A.L., Badjeck, M.-C., 2009. Vulnerability of national economies to the impacts of climate change on fisheries. FIsh Fish. 10, 173–196.

Alsop, D., Wood, C., 1997. The interactive effects of feeding and exercise on oxygen consumption, swimming performance and protein usage in juvenile rainbow trout (Oncorhynchus mykiss). J. Exp. Biol. 200, 2337–46.

Blazka, P., 1960. A new type of respirometer for the determination of the metabolism of fish in an active state. Physiol. Bohemoslov.

Bopp, L., Resplandy, L., Orr, J.C., Doney, S.C., Dunne, J.P., Gehlen, M., Halloran, P., Heinze, C., Ilyina, T., Séférian, R., Tjiputra, J., Vichi, M., 2013. Multiple stressors of ocean ecosystems in the 21st century: projections with CMIP5 models. Biogeosciences 10, 6225–6245. doi:10.5194/bg-10-6225-2013

Brandt, S.B., Gerken, M., Hartman, K.J., Demers, E., 2009. Effects of hypoxia on food consumption and growth of juvenile striped bass (Morone saxatilis). J. Exp. Mar. Bio. Ecol. 381, S143–S149. doi:10.1016/j.jembe.2009.07.028

Breitburg, D., Seitzinger, S., Sanders, J., 1999. Front Matter. Limnol. Oceanogr. 44, 1.

Brett, J.R., 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Canada 21, 1183–1226.

Claireaux, G., Lagardére, J.P., 1999. Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. J. Sea Res. 42, 157–168. doi:10.1016/S1385-1101(99)00019-2

Claireaux, G., Lefrançois, C., 2007. Linking environmental variability and fish performance: integration through the concept of scope for activity. Philos. Trans. R. Soc. B 362, 2031–2041. doi:10.1098/rstb.2007.2099

Claireaux, G., Webber, D.M., Lagardére, J.P., Kerr, S.R., 2000. Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (Gadus morhua). J. Sea Res. 44, 257–265. doi:10.1016/S1385-1101(00)00053-8

Crain, C.M., Kroeker, K., Halpern, B.S., 2008. Interactive and cumulative effects of multiple human stressors in marine systems. Ecol. Lett. 11, 1304–1315. doi:10.1111/j.1461-0248.2008.01253.x

Cruz-Neto, A.P., Steffensen, J.F., 1997. The effects of acute hypoxia and hypercapnia on oxygen consumption of the freshwater European eel. J. Fish Biol. 50, 759–769. doi:10.1006/jfbi.1996.0337

Denny, M., Helmuth, B., 2009. Confronting the physiological bottleneck: A challenge from ecomechanics. Integr. Comp. Biol. 49, 197–201. doi:10.1093/icb/icp070

Dewar, H., Graham, J.B., 1994. Studies of Tropical Tuna Swimming Performance in a Large Water Tunnel - Energetics. J. Exp. Biol. 192, 45–59.

Eliason, E.J., Clark, T.D., Hague, M.J., Hanson, L.M., Gallagher, Z.S., Jeffries, K.M., Gale, M.K., Patterson, D. a., Hinch, S.G., Farrell, a. P., 2011. Differences in Thermal Tolerance Among Sockeye Salmon Populations. Science (80-.). 332, 109–112. doi:10.1126/science.1199158

Farrell, A.P., Eliason, E.J., Sandblom, E., Clark, T.D., 2009. Fish cardiorespiratory physiology in an era of climate change. Can. J. Zool. 87, 835–851. doi:10.1139/Z09-092

Farrell, A.P., Hinch, S.G., Cooke, S.J., Patterson, D.A., Crossin, G.T., Lapointe, M., Mathes, M.T., 2008. Pacific Salmon in Hot Water: Applying Aerobic Scope Models and Biotelemetry to Predict the Success of Spawning Migrations. Physiol. Biochem. Zool. 81. doi:10.1086/592057

Fry, F.E.J., 1947. Effects of the environment on animal activity. Publ. Ontario Fish. Res. Lab. 55, 1–62.

Gaesser, G., Brooks, G., 1983. Metabolic bases of excess post-exercise oxygen consumption: a review. Med. Sci. Sports Exerc. 16, 29–43.

Hijmans, R.J., Graham, C.H., 2006. The ability of climate envelope models to predict the effect of climate change on species distributions. Glob. Chang. Biol. 12, 2272–2281. doi:10.1111/j.1365-2486.2006.01256.x

Hirzel, A.H., Le Lay, G., Helfer, V., Randin, C., Guisan, A., 2006. Evaluating the ability of habitat suitability models to predict species presences. Ecol. Modell. 199, 142–152. doi:10.1016/j.ecolmodel.2006.05.017

Hochachka, P.W., Darveau, C. a., Andrews, R.D., Suarez, R.K., 2003. Allometric cascade: A model for resolving body mass effects on metabolism. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 134, 675–691. doi:10.1016/S1095-6433(02)00364-1

Hoegh-Guldberg, O., Mumby, P.J., Hooten, a J., Steneck, R.S., Greenfield, P., Gomez, E., Harvell, C.D., Sale, P.F., Edwards, a J., Caldeira, K., Knowlton, N., Eakin, C.M., Iglesias-Prieto, R., Muthiga, N., Bradbury, R.H., Dubi, a, Hatziolos, M.E., 2007. Coral reefs under rapid climate change and ocean acidification. Science 318, 1737–1742. doi:10.1126/science.1152509

Homyack, J. a., 2010. Evaluating habitat quality of vertebrates using conservation physiology tools. Wildl. Res. 37, 332. doi:10.1071/WR08093

Hutchinson, G.E., 1957. Concluding remarks, in: Cold Spring Harbor Symposia on Quantitative BIology Vol. 22. Cold Spring Harbor Laboratory Press.

Kearney, M., Porter, W., 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. Ecol. Lett. 12, 1–17.

Khan, J.R., Pether, S., Bruce, M., Walker, S.P., Herbert, N.A., 2014. Optimum temperatures for growth and feed conversion in cultured hapuku (Polyprion oxygeneios) - Is there a link to aerobic metabolic scope and final temperature preference? Aquaculture 430, 107–113. doi:10.1016/j.aquaculture.2014.03.046

Killen, S.S., Costa, I., Brown, J.A., Gamperl, A.K., 2007. Little left in the tank: metabolic scaling in marine teleosts and its implications for aerobic scope. Proc. R. Soc. B. 274, 431–438. doi:10.1098/rspb.2006.3741

Killen, S.S., Mitchell, M.D., Rummer, J.L., Chivers, D.P., Ferrari, M.C.O., Meekan, M.G., McCormick, M.I., 2014. Aerobic scope predicts dominance during early life in a tropical damselfish. Funct. Ecol. In press. doi:10.1111/1365-2435.12296

Lambert, Y., Dutil, J.-D., 2000. Energetic consequences of reproduction in Atlantic cod (Gadus morhua) in relation to spawning level of somatic energy reserves. Can. J. Fish. Aquat. Sci. 57, 815–825. doi:10.1139/f00-022

Lapointe, D., Vogelbein, W.K., Fabrizio, M.C., Gauthier, D.T., Brill, R.W., 2014. Temperature, hypoxia, and mycobacteriosis: effects on adult striped bass Morone saxatilis metabolic performance. Dis. Aquat. Organ. 108, 113–27. doi:10.3354/dao02693

Le Quesne, W.J.F., Pinnegar, J.K., 2011. The potential impacts of ocean acidification: scaling from physiology to fisheries. Fish Fish. 1–12. doi:10.1111/j.1467-2979.2011.00423.x

Lefrançois, C., Claireaux, G., 2003. Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole Solea solea. Mar. Ecol. Prog. Ser. 259, 273–284.

Mallekh, R., Lagardére, J.P., 2002. Effect of temperature and dissolved oxygen concentration on the metabolic rate of the turbot and the relationship between metabolic scope and feeding demand. J. Fish Biol. 60, 1105–1115. doi:10.1006/jfbi.2002.1918

Mckenzie, D.J., Piccolella, M., Valle, A.Z.D., Taylor, E.W., Bolis, C.L., Steffensen, J.F., 2003. Tolerance of chronic hypercapnia by the European eel Anguilla anguilla. J. Exp. Biol. 206, 1717–1726. doi:10.1242/jeb.00352

Melzner, F., Göbel, S., Langenbuch, M., Gutowska, M.A., Pörtner, H.-O., Lucassen, M., 2009. Swimming performance in Atlantic Cod (Gadus morhua) following long-term (4-12 months) acclimation to elevated seawater P(CO2). Aquat. Toxicol. 92, 30–7. doi:10.1016/j.aquatox.2008.12.011

Moran, D., Støttrup, J.G., 2011. The effect of carbon dioxide on growth of juvenile Atlantic cod Gadus morhua L. Aquat. Toxicol. 102, 24–30. doi:10.1016/j.aquatox.2010.12.014

Munday, P.L., Crawley, N.E., Nilsson, G.E., 2009. Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. Mar. Ecol. Prog. Ser. 388, 235–242. doi:10.3354/meps08137

Nilsson, G.E., Crawley, N., Lunde, I.G., Munday, P.L., 2009. Elevated temperature reduces the respiratory scope of coral reef fishes. Glob. Chang. Biol. 15, 1405–1412. doi:10.1111/j.1365-2486.2008.01767.x

Plaut, I., 2001. Critical swimming speed: its ecological relevance. Comp. Biochem.

Physiol. A. Mol. Integr. Physiol. 131, 41–50.

Pörtner, H.-O., 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88, 137–146. doi:10.1007/s001140100216

Pörtner, H.-O., 2002. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 132, 739–61.

Pörtner, H.-O., 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. J. Exp. Biol. 213, 881–93. doi:10.1242/jeb.037523

Pörtner, H.-O., Berdal, B., Blust, R., Brix, O., Colosimo, A., De Wachter, B., Giuliani, A., Johansen, T., Fischer, T., Knust, R., 2001. Climate induced temperature effects on growth performance, fecundity and recruitment in marine fish: developing a hypothesis for cause and effect relationships in Atlantic cod (Gadus morhua) and common eelpout (Zoarces viviparus). Cont. Shelf Res. 21, 1975–1997. doi:10.1016/S0278-4343(01)00038-3

Reidy, S.P., Kerr, S.R., Nelson, J.A., 2000. Aerobic and anaerobic swimming performance of individual Atlantic cod. J. Exp. Biol. 203, 347–57.

Rijnsdorp, A.D., 1990. The mechanism of energy allocation over reproduction and somatic growth in female North Sea plaice, Pleuronectes platessa L. Netherlands J. Sea Res. 25, 279–289. doi:10.1016/0077-7579(90)90027-E

Schreck, C.B., 2010. Stress and fish reproduction: The roles of allostasis and hormesis. Gen. Comp. Endocrinol. 165, 549–556.

doi:10.1016/j.ygcen.2009.07.004

Schurmann, H., Steffensen, J.F., 1997. Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. J. Fish Biol. 50, 1166–1180. doi:10.1006/jfbi.1997.0387

Steffensen, J.F., 1989. Some errors in respirometry of aquatic breathers: how to avoid and correct for them. Fish Physiol. Biochem. 6, 49–59.

Stumpp, M., Wren, J., Melzner, F., Thorndyke, M.C., Dupont, S.T., 2011. CO2 induced seawater acidification impacts sea urchin larval development I: Elevated metabolic rates decrease scope for growth and induce developmental delay. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 160, 331–340. doi:10.1016/j.cbpa.2011.06.022

Thuiller, W., 2004. Patterns and uncertainties of species' range shifts under climate change. Glob. Chang. Biol. 10, 2020–2027. doi:10.1111/j.1365-2486.2004.00859.x

Vanella, F.A., Boy, C.C., Lattuca, M.E., Calvo, J., 2010. Temperature influence on post-prandial metabolic rate of sub-Antarctic teleost fish. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 156, 247–254. doi:10.1016/j.cbpa.2010.02.006

Weibel, E.R., Bacigalupe, L.D., Schmitt, B., Hoppeler, H., 2004. Allometric scaling of maximal metabolic rate in mammals: Muscle aerobic capacity as determinant factor. Respir. Physiol. Neurobiol. 140, 115–132. doi:10.1016/j.resp.2004.01.006

Widdows, J., Nasci, C., Fossato, V.U., 1997. Effects of pollution on the scope for growth of mussels (Mytilus galloprovincialis) from the Venice Lagoon, Italy. Mar. Environ. Res. 43, 69–79. doi:10.1016/0141-1136(96)00003-7

1.9. Figures and Tables

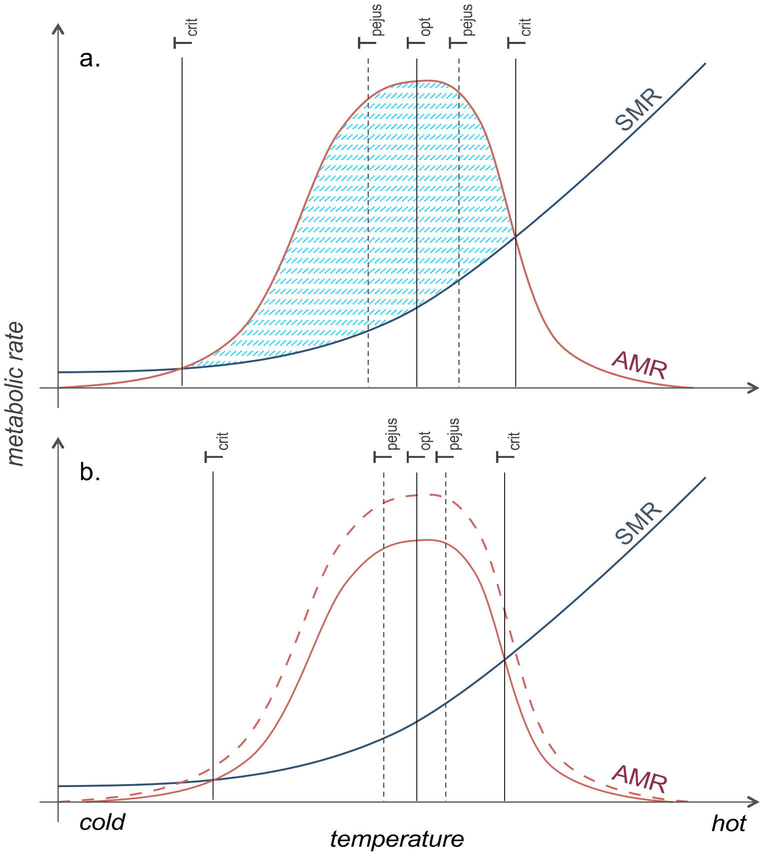

Figure 1: Schematic representation of changes in aerobic scope due to environmental perturbation. The temperature-limited thermal tolerance framework states that in case of temperature perturbation **(a),** standard metabolic rates (SMR; blue line) rise exponentially with increasing temperature (commonly described using a Q_{10} parameter of 2-3; Schurmann and Steffensen, 1997) while active metabolic rates (AMR; solid red line) follow a bell-shaped trend. The difference between the active and standard metabolisms is the aerobic scope (blue shaded region). Aerobic scope is largest at a temperature optimum (Topt) but exhibits a relatively stable trend up to a threshold on either side of the optimum (Tpejus) where aerobic scopes become more acutely sensitive to temperature perturbation. Long-term habitation becomes

impossible under temperatures beyond the critical limits (Tcrit), which describe the limits of an above-zero aerobic scope. In the case of simultaneous stressors such as low oxygen (hypoxic stress) or high pCO_2 (hypercapnic stress) **(b)**, a decline in active metabolic rate (dotted red line: unstressed; solid red line: stressed) often results in a narrowing of critical and pejus temperature limits and a decrease in the maximum active metabolic rate without changing the optimum temperature (T_{opt}). Figure adapted from Pörtner (2001).

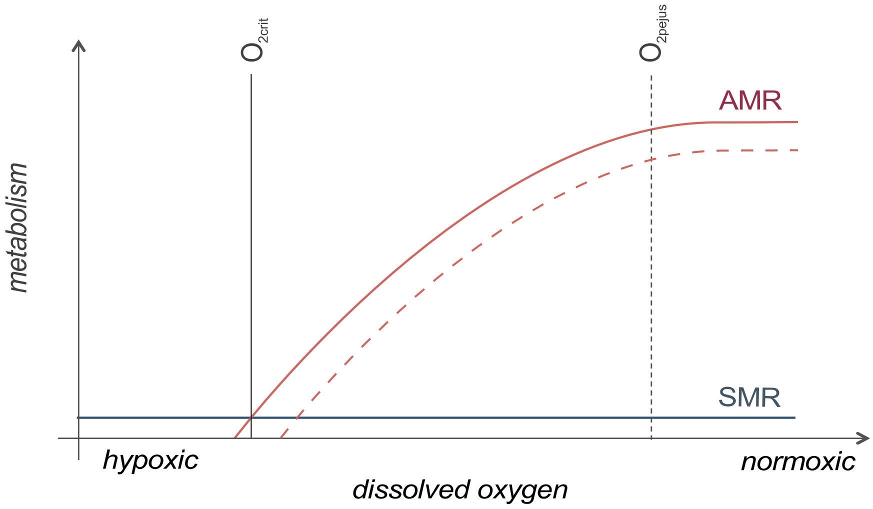

Figure 2: Change in aerobic scope in response to dissolved oxygen perturbation. The limiting oxygen concentration framework posits a maximum active metabolic rate (AMR; solid red line) at a high oxygen concentration (oxygen saturation = 100%) with declining oxygen concentrations resulting in an exponential decrease in active metabolism. O_{2pejus} and O_{2crit} are analogous to T_{pejus} and T_{crit} in Figure 1. Simultaneous stressors such as high temperature (hyperthermia) or high pCO_2 (hypercapnia) lead to a decline in maximum active metabolic rate (dotted red line) without changing the optimum dissolved oxygen concentration. Figure adapted from Claireaux and Lagardére (1999).

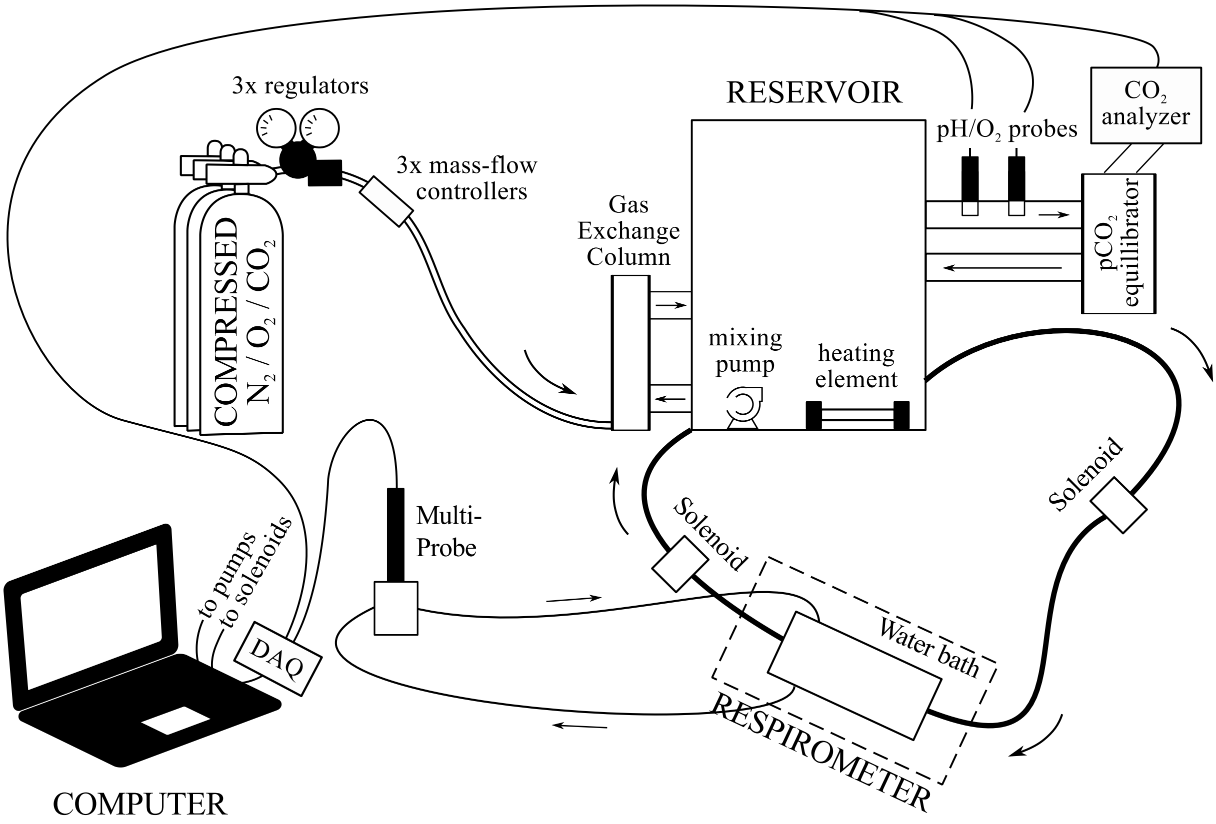

Figure 3: Schematic diagram of climate-controlled swim tunnel respirometer system. A closed-feedback sensor loop controls the flow rate of N_2, O_2 and CO_2 gas into a counter-current exchange column to set the gas tensions in a large UV-sterilized reservoir. Solenoids control the flow of water from the reservoir to the respirometers, which are maintained at constant temperature by a water bath. A dissolved oxygen optode, pH electrode, and temperature probe housed in a flow-through cell measures water conditions in the respirometer (Thermo Fisher Scientific, Waltham MA). The solenoids, gas flow rate, and sealing and unsealing of the respirometer are all fully automated through custom software implemented on a PC coupled with electronic controllers (Neptune Systems, Morgan Hill CA) with PID (proportional-integrative-derivate) software.

Table 1: Linear correlations between aerobic scope and fitness-related activities in captive teleosts. Significance denotes the p-value of a linear regression.

Parameter	**Species**	**Significance**	**R^2**	**Reference**
Specific Growth Rate	*Dicentrarchus labrax*	< 0.0001	0.975	(Claireaux and Lefrançois, 2007)
Specific Growth Rate	*Polyprion oxygeneios*	0.03	0.92	(Khan et al., 2014)
Specific Growth Rate	*Gadus morhua*	<0.01	0.847	(Claireaux et al., 2000)
Daily Feeding Rate	*Scophthalmus maximus*	<0.01	0.998	(Reidy et al., 2000)
Critical Swimming Speed	*Gadus morhua*	<0.05	0.525	(Mallekh and Lagardére, 2002)
Territorial Aggression	*Pomacentrus amboinensis*	0.001	0.267	(Killen et al., 2014)

Table 2: Comparison of predictive frameworks for forecasting the effect of environmental conditions on species occurrence, abundance, or performance. After Kearney and Porter (2009).

	Mechanistic/experimental	**Correlative/statistical**

	Approaches	**Advantages**	**Approaches**	**Advantages**
Model selection	Prescribed (e.g. energy balance equation)	Physiological traits provide common frame of reference	Flexible (e.g. generalized linear regression, maximum entropy)	More likely to fit available data
Generality	High across environments	Applies to non-equilibrium/novel environments	Local application	Less likely to overestimate potential range
Data requirement	Functional traits (physiology or behavioral responses)	Direct link between physiology and species range	Occurrence data (e.g. presence only, presence/absence, abundance)	Data source is commonly available
Scale	Individual organism	Less subjectivity in model selection	Flexible	Can capture processes across a range of scales
Genetic variability	Explicit	Can assess the degree of	Implicit	Geographic variation already

and physiological plasticity		adaptive capacity		incorporated in occurrence data
Output	Fitness components (e.g. growth, performance, reproductive capacity	Highly ecologically interpretable	Dimensionless habitat suitability indices or probability of occurrence or abundance	Single output indirectly represents many different processes
Model evaluation	Usually evaluated against independent datasets (e.g. occurrence/abundance in the field, field or laboratory measurements of physiology)	Can more easily evaluate against independent datasets	Evaluated against original input data, subsets of original data set aside for evaluation (i.e. cross-validation)	Convenient because model construction/evaluation relies on the same data source

2. An Aerobic Scope-Based Habitat Suitability Index for Predicting the Effects of Multi-Dimensional Climate Change Stressors on Marine Teleosts

2.1. Abstract

Climate change will expose many marine ecosystems to temperature, oxygen and CO_2 conditions that have not been experienced for millennia. Predicting the impact of these changes on marine fishes is difficult due to the complexity of these disparate stressors and the inherent non-linearity of physiological systems. Aerobic scope (the difference between maximum and minimum aerobic metabolic rates) is a coherent, unifying physiological framework that can be used to examine all of the major environmental changes expected to occur in the oceans during this century. Using this framework, we develop a physiology-based habitat suitability model to forecast the response of marine fishes to simultaneous CO_2-induced ocean acidification, warming and deoxygenation, including interactions between all three stressors. We present an example of the model parameterized for *Thunnus albacares* (yellowfin tuna), an important fisheries species that is likely to be affected by climate change. We anticipate that if embedded into multispecies ecosystem models, our model could help to more precisely forecast climate change impacts on the distribution and abundance of other high value species. Finally, we show how our model may indicate the potential for, and limits of, adaptation to chronic stressors.

2.2. Introduction

2.2.1. Habitat quality is central to conservation biology

Habitat alteration is the dominant driver of species extinction in our time (Homyack, 2010) and is likely to only increase in importance in the future due to the perturbations brought about by global climate change (Thomas et al., 2004; Travis, 2003). The ubiquity of habitat quality models (i.e. those that attempt to simulate species distribution based on habitat variables) across different disciplines of ecology reflects this importance. For example, one of the simplest forms of the habitat quality model is the climate envelope model (CEM), which correlates presence/absence data against the spatial distribution of environmental variables to try to define the limits of a species distribution (Thomas et al., 2004). A generalized form of the CEM is the habitat suitability model, which attempts to simulate not only the limits of a species' distribution but also its probability of occurrence/abundance within its range (Hirzel and Le Lay, 2008).

These habitat suitability models can be incorporated into more complex models either as a single-species add on to a fisheries assessment model (Link et al., 2011) or as part of a niche model that adds bionomic factors (resource limitation or predation pressures that define a Grinellian niche) through interactions with other species whose distribution and abundance is partially driven by habitat suitability models incorporating scenopoetic factors (e.g. temperature and oxygen limits that define an Eltonian niche) (Hutchinson, 1957). When enough niche models are aggregated to represent the full breadth of trophic levels existing in an ecosystem, these are then termed end-to-end ecosystem models (see Lehodey et al., 2008; Link et al., 2010; Maury, 2010) and may be used to perform large-scale simulations of ecosystem-responses to climatic or biogeochemical shifts (Lehodey et al., 2010).

Finally, a special application of habitat suitability models are those required by fisheries biologists who need to disentangle habitat effects from stock abundance in order to constrain their estimates of optimum yield. These so-called habitat-based standardizations (Hinton and Nakano, 1996) generally take the form of correction factors to a “catchability” term that are used to ensure that the catch per unit effort at a given location and time is more reliably indicative of abundance (Maunder et al., 2006).

2.2.2. Physiology is a key constraint for climate-change studies

While all of the model types discussed above can be applied both to current and future environments, habitat suitability models that attempt to tackle the problem of climate change face fundamentally different challenges than those that are primarily concerned with current or historical environments. This is because applying habitat suitability models to a climate change future is inherently extrapolative – that is, future habitats are likely to involve either novel states (e.g. temperature or pCO_2 levels not previously experienced in a given region) or novel combinations of states (e.g. unusually high temperatures combined with low oxygen) for which species distribution data is not currently available (Kearney and Porter, 2009). The inherent non-linearity of physiological control mechanisms (typically involving some tolerable range of environmental stressors bounded by *pejus* limits beyond which fitness rapidly declines (Nagarajan, 2002; Pörtner, 2002)) and the fact that the regulatory machinery involved in responding to distinct stressors often overlap (leading to complex interactions when multiple environmental variables are perturbed simultaneously; see (Claireaux and Lagardére, 1999; Kato et al., 2005; Lefrançois and Claireaux, 2003; Perry and Gilmour, 1996; Roch and Maly, 1979)) make these extrapolations highly uncertain. To make matters worse, many of the environmental variables used to inform habitat suitability models (e.g. temperature, oxygen, pCO_2)

exhibit high spatial correlation (Prince and Goodyear, 2006; Schmittner et al., 2008), compromising the ability to assign a gradient in species abundance to a specific variable. Indeed, many studies have demonstrated large variability in estimated abundance when extrapolating statistical models trained on current observations to future states (Thuiller, 2003; Thuiller, 2004; Thomas et al., 2004; Hijmans and Graham 2006).

One way to ameliorate these problems is to constrain the results of statistical habitat suitability models with information from organismal physiology (Denny and Helmuth, 2009; Hijmans and Graham, 2006). Physiological relationships provide a mechanistic (or semi-mechanistic) basis for understanding changes in abundance for which (in the absence of substantial genetic or epigenetic adaptation) we can assume stationarity into the relatively distant future (Kearney and Porter, 2004). Furthermore, because organismal physiology is principally modified by genetic or epigenetic processes, physiology-based models address a major weakness in statistical habitat suitability models by allowing us to ask questions such as (1) which physiological parameters could play a role in mitigating the changes to habitat suitability caused by climate change, and (2) is the currently observed variability in these parameters (indicative of the range of phenotypic plasticity) sufficient to adapt to future climate change even in the absence of genotypic or epigenetic modification (Kearney and Porter, 2009)?

2.2.3. Aerobic scope is an integrative index of abiotic habitat suitability

Implicit in the concept of linking organismal physiology to habitat suitability (and, after consideration of bionomic factors, to abundance) is the criteria that the metric of physiological state used must be linked to fitness (Homyack, 2010). The most popular methods utilize some measure of energetic state, such as the metabolic cost of key fitness-determining behaviors including digestion, hibernation, flight, and foraging; or of benchmark metabolic states such as standard (minimum) or active (maximum) metabolism (Braaten et al., 1997; Homyack, 2010; Rosenfeld et al., 2005). In this paper we utilize aerobic scope (the difference between maximal aerobic metabolism and the minimum metabolic expenditure needed for survival (Fry, 1947)) as an integrative index of habitat suitability with strong ties to fitness. Because the machinery that underlies aerobic metabolism is conserved across metazoan taxa (Thannickal, 2009), much of the discussion below is broadly applicable.

The correspondence between aerobic scope and the other energetic indicators discussed above can be seen by partitioning an organism's energy budget into four parts: (1) the proportion of total energy required to maintain essential life processes, (2) the proportion that can be devoted to foraging and somatic growth, (3) the proportion dedicated to reproduction either in the form of gonadal growth or activities such as maintaining a territory, and (4) the energy available for storage (Homyack, 2010). In vertebrates, almost all of these energetic costs must be paid for through aerobic metabolism (Pörtner, 2010), either by directly powering these activities or via excess post-exercise oxygen consumption (EPOC: Gaesser and Brooks, 1983; Lee et al., 2003). The aerobic scope can be thought of as the total potential metabolic expenditure available for fueling portions 2-4 in excess of that required for portion 1. Reductions in aerobic scope either through an increase in minimum metabolism (a rise in portion 1) or a decrease in maximum metabolism (a decline in the total available energy output) therefore imply a reduction in the maximum rate for one of the processes (growth, reproduction, or energy storage) that drives current and future fitness. Furthermore, aerobic scope defines physical limits to long-term habitation at the critical limits (conditions under which aerobic scope is zero) beyond which maintenance of essential life processes is only possible by drawing down stored oxygen reserves or relying (temporarily) upon anaerobic capacity (Pörtner, 2010). This definition is only a slight variation on what is termed the fundamental niche: the space where, in the absence of biotic interactions, an organism can survive and reproduce (Kearney and Porter 2004). These properties make aerobic scope a strong metric on which to base a physiological habitat-suitability model.

2.2.4. Thunnus albacares as a model organism for aerobic scope modeling

While a number of species could conceivably be used to parameterize an aerobic scope model, we chose the yellowfin tuna *Thunnus albacares* for this study due to the wealth of physiological data available on this species as well as the multiple environmental changes that are likely to befall portions of its habitat due to climate change. These include increased surface ocean temperatures, surface ocean and thermocline deoxygenation, and increased hypercapnia (high levels of pCO_2) both in the surface ocean and especially in the thermocline and oxygen minimum zones where high levels of dissolved carbon dioxide have already substantially depleted the buffering capacity of seawater (Brewer and Peltzer, 2009; Cai et al., 2011; Gruber, 2011a). We rely on well-established physiological equations to create an aerobic scope estimator from already available laboratory data, and incorporate both the mean and variability of this data to create not just an estimate of how these complex environmental perturbations could affect habitat suitability for *T. albacares* but also some indications of the extent to which the observed variability in these parameters could compensate for future climate change.

While this paper provides one example of an aerobic-scope based habitat suitability model parameterized for a single species at a single location, we have designed this model to be applicable to a wide range of teleosts in many different marine environments with very little modification, and we hope broader aspects of the model can be useful for an even wider range of taxa.

2.3. Methods

We developed a three-box model of a tuna consisting of a conceptually infinite seawater reservoir, a gill reservoir, and a reservoir for the working tissues (principally the muscles). The maximum metabolic rate model consists of ten coupled equations drawn from classical physiological relationships (e.g., the Fick Equation, the Van't Hoff Equation, the Bohr effect) and empirical regression equations (e.g., temperature and oxygen effects on cardiac output), from which we subtract an empirically derived estimate of minimum metabolic rate to obtain the aerobic scope. In the maximum metabolic rate model, the flux of blood between the gills and working muscles is determined by the cardiac output (the rate of blood flow out of the heart), and the rate of oxygen flux per unit of blood volume is determined by modeled red blood cell kinetics, which is in turn affected by oxygen concentration, temperature, and blood pH.

Vertebrate red blood cells are extremely complicated physiological systems with many effectors. The effects of allosteric molecules such as adenosine triphosphate (ATP) and guanosine triphosphate (GTP), as well as the effects of catecholamine release driving the activity of Na+/H+ exchangers in the red blood cell membranes have not been explicitly modeled in these equations even though they can have strong effects on oxygen binding. Both nucleoside triphosphates and circulating catecholamines can increase dramatically in fishes during maximal exercise and in other aerobically stressful conditions (Berenbrink and Bridges, 1994; Lowe and Brill, 1998). Our approach to this issue was to parameterize our equations as much as possible from data derived from experimental animals undergoing maximal exercise under the assumption that these parameters more closely reflected the kinetics of oxygen binding under conditions of maximal NTP and catecholamine release (e.g. Korsmeyer et al (1997)). The full list of parameter names, symbols, and chosen values are reported in Tables 1 and 2.

2.3.1. Red blood cell kinetics

To approximate the effects of oxygen partial pressure, temperature and pH on the oxygen content of the blood, we begin by expressing arterial and venous oxygen content (O_a and O_v, respectively) as a function of red blood cell hemoglobin oxygen saturation (S) and the red blood cell oxygen capacity (κ) (Korsmeyer et al., 1997)

$$O_a = S_a \kappa, \qquad O_v = S_v \kappa \tag{2.3-1}$$

where κ is the product of the mean corpuscular hemoglobin concentration ($\overline{H}$), the hemoglobin oxygen binding capacity (O_H) and the hematocrit (Hct)

$$\kappa = \overline{H} \cdot O_H \cdot Hct \tag{2.3-2}$$

The red blood cell hemoglobin oxygen saturation can in turn be expressed as a function of the oxygen partial pressure (pO_2), the half-saturation constant ($p50$), and the hill coefficient of cooperativity (η) (Hill, 1910)

$$S = \frac{pO_2{}^{\eta}}{pO_2{}^{\eta} + p50^{\eta}} \tag{2.3-3}$$

We then model the effect of temperature and pH on the half-saturation constant by combining the Bohr (Siggaard-Andersen and Garby, 1973) and Van't Hoff equations (Brill and Bushnell, 1991)

$$\log p50(\Delta T, \Delta pH) = \log p50_0 + \varepsilon_T + \varepsilon_{pH} \qquad (2.3\text{-}4)$$

$$\varepsilon_T = \frac{H[\Delta(T^{-1})]}{2.303R} \qquad \varepsilon_{pH} = \Delta pH \cdot \beta \qquad (2.3\text{-}5)$$

where R is the ideal gas constant (1.987 cal K^{-1} mol^{-1}), H is the apparent heat of hemoglobin oxygenation and β is the extracellular fixed-acid Bohr coefficient ($\Delta \log p50/\Delta pH$).

Solving equation (2.3-1) under the pH, pO_2, and temperature conditions of the arterial and the venous bloodstreams allows us to calculate the arterio-venous oxygen difference (ΔO) as simply

$$\Delta O = O_a - O_v \qquad (2.3\text{-}6)$$

2.3.2. Blood carbonate chemistry

To solve the carbonate system in the arterial and venous blood, we began by making the following assumptions:

i. The pCO_2 of the arterial bloodstream (pCO_{2a}) is equal to the pCO_2 of the inspired seawater. This condition approximates the condition for water-breathing animals, which are unable to regulate pCO_{2a} through ventilatory processes (Cameron and Polhemus, 1974) and (in the absence of metabolic acidosis arising from significant anaerobic energy output) are generally unable to maintain substantial pCO_2 gradients across the gill membranes (Ishimatsu et al., 2004; Toews et al., 1983).

ii. The pH of the arterial bloodstream (pH_a) is constant in the face of environmental hypercapnia. This assumption follows from experimental results showing a complete compensation of arterial pH within 12-24 hours in the face of acute hypercapnia up to and including lethal levels (Ishimatsu et al., 2005).

iii. The temperature of the arterial bloodstream (T_a) equals the temperature of the inspired water, while the temperature of the venous bloodstream (T_v) equals the temperature of the arterial bloodstream plus a constant term (T_{excess}) reflecting the effect of regional endothermy (in certain teleosts including some in the genus *Thunnus*) on the temperature of the working muscles.

iv. The venous dissolved inorganic carbon concentration (C_v) is equal to the arterial dissolved inorganic carbon concentration (C_a) plus the moles of oxygen produced through metabolism (i.e. ΔO) due to the stoichiometry of the cellular respiration equation assuming a respiratory quotient (RQ) of 1. An RQ of one, implying that metabolic output is dominated by carbohydrate oxidation (as opposed to fats or proteins) driving aerobic metabolism, is appropriate for states of maximal aerobic output where the rates of fat and protein catabolism are too slow to make a significant contribution (Kutty, 1968; Richardson, 1923).

v. The venous oxygen partial pressure (pO_{2v}) at maximal exercise approaches a constant value (the minimum venous oxygen partial pressure). This assumption derives from the fact that exercising venous pO_2 is relatively invariant in a wide variety of animals (Farrell, 2002), as is the case for *T. albacares* (Bushnell, 1988). It is important to note however that this does not preclude changes to venous oxygen *content* (O_v) due to fluctuations in the pH or temperature of the venous bloodstream. In this study, we do not take into account the collapse of pO_{2v} beyond the minimum limits which can lead to acute cardiorespiratory collapse and reduced neural functioning (Farrell, 2002) because these values occur beyond the pejus ranges we are considering.

vi. The arterial oxygen partial pressure (pO_{2a}) is equal to the pO_2 of the inspired water multiplied by a utilization constant (U) where $0 < U \leq 1$.

With these assumptions, we solve for arterial DIC and total alkalinity (A_a) given arterial pH, pCO_2 and temperature using the R package seacarb (Lavigne et al., 2014).

2.3.3. *Arterio-venous oxygen difference*

We initially assume a constant DIC ($C_v = C_a$) and the absence of an arterio-venous oxygen difference ($\Delta O = 0$). We then iterate through the equations

$$\Delta O_i = O_a - O_{v_i}\left(C_{v_i}, T_v, pO_{2v}\right) \tag{2.3-7}$$

$$C_{v_{i+1}} = C_v + \Delta O_i \tag{2.3-8}$$

with the condition $\Delta O_i \nless 0$.

The stable value (ΔO_n), defined operationally as $\Delta O_{i+1} - \Delta O_i < 10^{-7}$ mol kg^{-1} is then taken to be the steady-state arterio-venous oxygen difference for a given set of environmental parameters.

2.3.4. *Cardiac Output*

To characterize the maximum cardiac output ($\dot{Q}$) under environmental perturbation we first made the following assumptions about the temperature (T_h) and oxygen (O_h) state of the heart

$$T_h = T_a, \qquad O_h = fO_a + (1-f)O_v \tag{2.3-9}$$

where $0 \leq f \leq 1$. In all teleosts including regional endotherms such as tuna, the position of the heart upstream of the rete mirabile means that cardiac temperature is indistinguishable from the temperature of the arterial circulation (and therefore from the temperature of the inspired seawater). Oxygen supply to the heart however shows marked variation between taxa. In many teleosts, the myocardial oxygen supply derives entirely from the venous circulation (Farrell, 2002), corresponding in the model to a situation where $f = 0$. More athletic fishes typically possess a coronary circulation that supplies arterial blood to the compact myocardium. In salmonids for example it is estimated that the coronary vasculature supplies about 30% of peak cardiac demand ($f \cong 0.3$) (Farrell, 2002). Tunas such as *T. albacares* have developed an even more extensive coronary circulation capable of supplying the majority of cardiac oxygen demand (Farrell, 1996), and we set $f = 1$ in this case.

We characterized the maximum $\dot{Q}$ using an empirically determined two-piece linear function (analogous to that used by Bigelow and Maunder (2007)) where temperature and oxygen were assumed to have a constant effect on $\dot{Q}$ below a threshold value (O_T, T_T) and no effect above it.

$$\dot{Q}(O_h, T_h) = Q_{max}\left[\frac{\delta\dot{Q}}{\delta O_h}(O_h - O_T) + \frac{\delta\dot{Q}}{\delta T_a}(T_h - T_T)\right] \tag{2.3-10}$$

$$\frac{\delta\dot{Q}}{\delta O_h} = \begin{cases} \lambda_1, & O_h < O_T \\ 0, & O_h \geq O_T \end{cases}, \qquad \frac{\partial\dot{Q}}{\partial T_h} = \begin{cases} \lambda_2, & T_h < T_T \\ 0, & T_h \geq T_T \end{cases} \tag{2.3-11}$$

where Q_{max} is the maximum possible cardiac output under optimum conditions and λ_1 and λ_2 are the magnitudes of the oxygen and temperature sensitivity, respectively.

2.3.5. *Aerobic scope*

We calculate maximum aerobic metabolism ($\dot{M}_{max}$) by combining equations (2.3-6) and (2.3-10) into the Fick equation (Selzer and Sudrann, 1958)

$$\dot{M}_{max} = \dot{Q}(\Delta O) \tag{2.3-12}$$

The minimum metabolic rate ($\dot{M}_{min}$) is then estimated according to a commonly used empirical regression

$$\dot{M}_{min} = c\mathrm{F}^{0.1\Delta T} \tag{2.3-13}$$

In the case of *T. albacares*, we used the regression parameters calculated by Dewar and Graham (1994) for tuna of mass 2.17 kg (fork length 51.3 cm) corrected to the minimum hydrostatic swimming speed calculated by Magnuson (1973) to obtain a routine metabolic rate. The mass was chosen to be similar to the electronically tagged fish for which depth distribution data could be obtained (Schaefer et al., 2007). The routine metabolic rate correction was used to provide a more realistic minimum survivable metabolic rate than would standard metabolism, which is defined as metabolism in the absence of growth, digestion or locomotion and would constitute a lethal metabolic expenditure in obligate ram ventilators such as tunas (Bushnell and Brill, 1991; Dotson, 1976).

Finally, aerobic scope ($\dot{M}_{scope}$) is obtained by subtracting equation (2.3-13) from (2.3-12)

$$\dot{M}_{scope} = \dot{M}_{max} - \dot{M}_{min} \tag{2.3-14}$$

2.3.6. Statistical Methods

Uncertainty estimates for the aerobic scope were obtained using latin-hypercube sampling based Monte Carlo error propagation (samples per parameter = 1000). Throughout this paper, uncertainty estimates reflect both the range of physiological parameter values (e.g. variability in the measured Bohr coefficient β) and the variability in environmental conditions (e.g. water temperature at depth). In all cases, input parameters were assumed to be normally distributed with mean and standard deviations calculated from experimental data.

Sensitivity metrics were estimated using Sobol global variance-based sensitivity analysis (Saltelli et al., 2010) using the Python package SALib (Herman et al., 2014) to determine both first-order main effects (the magnitude of uncertainty of the output variable resulting from uncertainty in the input parameter) and total effects (main effects + interaction effects).

2.3.7. Model Evaluation

To evaluate the aerobic scope estimate as an indicator of habitat suitability, we constructed a simple linear estimator of vertical habitat usage

$$f_i = y\dot{M}_{scope_i} + b \qquad (2.3\text{-}15)$$

where f_i is the percentage of total time that a fish would be expected to spend at depth i, $\dot{M}_{scope_i}$ is the output of the aerobic scope model evaluated at the environmental conditions ($temperature,\ pO_2, pCO_2$) for that depth, and y and b are fitting parameters. To isolate the scenopoetic influence on habitat choice and minimize any bionomic effects (e.g. the availability of prey, competition from other predators) we focused on a study area with strong gradients in environmental variables driving pronounced vertical habitat compression, which for *T. albacares* was chosen to be the Eastern Tropical Pacific oxygen minimum zone, with further focus on the region bounded by the latitudes 5°S-5°N and the longitudes 90-105°W (the study area) for which electronic-tagging data on vertical habitat usage was presented in Schaefer et al. (2007).

2.4. Results

2.4.1. Interactions between environmental variables

The aerobic scope model revealed multiple putative interactions between environmental variables (Figure 4). The effects of temperature perturbations on $\dot{M}_{scope}$ showed the largest amplification under conditions of high pO_2, low temperature, and low pCO_2, with the sign of the temperature effect ($\partial\dot{M}_{scope}/\partial T$) being positive below the threshold temperature O_T (increasing temperature increases $\dot{M}_{scope}$) and negative above (increasing temperature decreases $\dot{M}_{scope}$). Oxygen perturbations ($\partial\dot{M}_{scope}/\partial pO_2$) were always positive and most influential at the threshold oxygen concentration (O_T), threshold temperature (T_T) and low pCO_2. Finally, pCO_2 effects ($\partial\dot{M}_{scope}/\partial pCO_2$) were always negative and relatively invariant across different pO_2, but amplified at high pCO_2 and high temperature.

2.4.2. Defining projected climate change perturbations

Recent historical data for the study area were obtained from the World Ocean Atlas 2013 (Garcia et al., 2014; Locarnini et al., 2013) and the Global Ocean Data Analysis Project (Key et al., 2004) for the depths 0-325 m (Figure 5). These data showed substantial changes in temperature, pO_2 and pCO_2 within the upper 100 m, with the depth profiles being divisible into a surface mixed layer (0-35 m depth), a thermocline layer (35-80 m depth), and an oxygen minimum layer (80-300 m). Representative mean conditions in the three layers were: mixed layer (Temperature

296 K; pO_2 0.19 atm; pCO_2 410 µatm), thermocline (Temperature 291 K; pO_2 0.12 atm; pCO_2 510 µatm), and oxygen minimum layer (Temperature 287 K; pO_2 0.07 atm; pCO_2 620 µatm). Comparison of these values against projections for end-century environmental conditions obtained from the Meteorological Research Institute-Earth System Model Version 1 (MRI-ESM1; (Yukimoto et al., 2011)) under the RCP 8.5 emissions scenario suggest that our study area will undergo a suite of perturbations over the next century, including a 2-5 K increase in mean temperatures, an approximately 20,000 µatm increase in mixed layer pO_2 coupled with an up to 30,000 µatm decline in the oxygen minimum layer, and a pCO_2 increase ranging from 700 µatm at the surface to 2,800 µatm at depth.

2.4.3. Aerobic scope as a predictive variable

Predicted aerobic scope was obtained for the study region by Monte Carlo sampling of both historical environmental variables (using the data sources described in section 2.4.2) and physiological parameters (the means and standard deviations are listed in Table 4). We tested the utility of the predicted aerobic scope as an indicator of habitat suitability by regressing predicted aerobic scope against the observed vertical distribution of 5 electronically tagged *T. albacares* in the same region (Schaefer et al., 2009). The resulting correlation (Figure 6) shows some autocorrelation of residuals but also a tight ($R^2 = 0.91$) and highly significant ($p < 0.0001$) linear fit, with the value of the regression coefficients in equation (3.7-1) estimated as $y = 2.79$, $b = 5.10$.

2.4.4. Projected changes in vertical habitat

Using the linear regression model of vertical distribution obtained in 2.4.3, we projected changes in vertical habitat between current and end-century conditions in the Galapagos region (Figure 7). Our analysis showed vertical habitat compression driven by the changing environmental variables in the thermocline resulting in the shoaling of the critical limits (environmental conditions causing aerobic scope to fall to zero) from 90 m in the current ocean, to 60 m by the end of the century. By allowing the input physiological parameters to vary in a bootstrapping scheme (using the mean and standard deviations reported in Table 4), we were able to isolate the 95th percentile of results for which the estimated aerobic scope in the end-century scenario was closest to the present. This analysis showed that the observed range of physiological plasticity could mitigate the effects of climate change perturbations in the upper thermocline (40-65 m) but is unlikely to mitigate impacts in deeper waters.

2.4.5. Sensitivity analysis

A Sobol global variance-based sensitivity analysis revealed a large range in model sensitivities to the input physiological parameters for *T. albacares* (Figure 8), with temperature and oxygen related parameters ($O_{utilization}, O_{slope}$) and the magnitude of regional endothermy (T_{excess}) having the strongest effect. In all cases except for the temperature threshold of cardiac output (T_T) and the slope of the temperature response (λ_2), the analysis showed a substantially (3-9 fold) higher interaction effect compared to the main effect.

2.5. Discussion

2.5.1. Aerobic scope as a habitat suitability index

Our results demonstrate a habitat suitability model capable of integrating multivariate climate-change related perturbations into a unified physiological framework. Using input parameters representative of a generalized marine teleost, we revealed that our model includes multiple putative interactions between simultaneous environmental stressors that are likely to be lacking in correlative habitat suitability models. This is particularly important when considering that climate change perturbations are generally multivariate (Gruber, 2011b; Prince and Goodyear, 2006) and that complex interactions between stressors (including synergisms and antagonisms) are ubiquitous in ecological systems (Crain et al., 2008). The complex changes projected to occur by the end of the century in our study region in the Eastern Tropical Pacific exemplify these trends, with each of the different depth zones (mixed layer, thermocline, and oxygen minimum layer) likely to experience qualitatively distinct perturbations over the next 100 years. Using input parameters specific to one teleost (*T. albacares*), we create a simple linear model to predict vertical habitat usage from aerobic scope and demonstrate that this estimator has high fidelity to the actual habitat usage observed through electronic tagging of 5 individuals in the study region. Furthermore, we project that these changes could manifest as a pronounced vertical habitat compression in this region. Through Monte Carlo sampling from the distribution of measured physiological parameters, we not only estimate uncertainty distributions for this vertical habitat usage, we also derive an estimator of adaptive capacity and predict that at least some of the projected habitat compression for *T. albacares* will be unaffected by adaptation operating through selection pressures alone.

2.5.2. Consequences of vertical habitat compression

Projected vertical habitat compression in the study area portends declining *T. albacares* abundance in the Eastern Tropical Pacific oxygen minimum zone in the future. This declining abundance may be exacerbated by a rise in catchability as the squeezing of the tuna population into a shallower depth range makes them more accessible to the longline and purse-seine fisheries operating in this region (Prince and Goodyear, 2006). In particular, surface-floating fish-aggregating devices (FADs), which are widely used between 15°S-5°N (Schaefer and Fuller, 2004) could interact more frequently with increasingly surface-restricted tunas. While we focus on one section of the eastern tropical pacific oxygen minimum zone near the Galapagos islands for which tagging data happened to be available, similar environmental pressures are likely to lead to habitat compression across much of the rest of the oxygen minimum zone, including the nearby and highly productive Costa Rican Dome ecosystem. Any changes will have a disproportionate impact on global *T. albacares* landings because of the unusually large tuna yield from this region (Essington et al., 2002).

Interestingly, our model suggests that part of the current depth distribution of *T. albacares* may already lie outside the critical limits set by environmental variables. This is consistent with studies on other epipelagic predators associated with oxygen minimum zones such as *Dosidicus gigas* (Rosa and Seibel, 2008), and implies that *T. albacares* spend a substantial portion of their time (18%) making temporary forays into habitats that require them to rely on some combination of blood oxygen stores and anaerobic metabolism (paid for by elevated metabolic rates upon return to more favorable surface waters). The ability of some tagged *T. albacares* to move deep into physiologically inhospitable environments including at least one dive to 1160 m and an ambient water temperature of 5.8°C (Dagorn et al., 2006) indicates that these adaptations may be highly developed. Our analysis suggests that *T. albacares* populations in the area will be forced to rely more frequently on these adaptations in the future.

2.5.3. Methods for integrating bionomic factors into our model

In our limited demonstration we assumed that aerobic scope (driven only by scenopoetic factors) was the dominant driver of habitat usage in the study region. This assumption was based on the existence of strong, persistent environmental gradients in the Eastern Tropical Pacific resulting in substantial vertical habitat limitation for tunas (Schaefer et al., 2009). While the remarkable tightness of fit of our model (R^2 was greater than 0.9) against habitat usage patterns observed in tagged animals is consistent with this assumption, there is noticeable autocorrelation of residuals, and it is likely that a parabolic or other nonlinear estimator could be more appropriate in future studies. Other possible sources of uncertainty include differences between the actual environmental conditions experienced by the tagged fish and the assumed conditions drawn from the WOA and GLODAP climatology, or to the contribution of minor variables that were not accounted for in the model. The most obvious candidates for these minor variables are bionomic factors.

More sophisticated analyses attempting to account for these factors, especially in regions with permissive environmental conditions, could easily incorporate the effect of additional bionomic terms. One term that is particularly straightforward to implement is prey availability, which can be conceptualized as a limiting factor that converts between the potential metabolic output (set by the aerobic scope) and the portion of that output that is consistent with the rate of energy input (prey availability). For example, a simple habitat preference model incorporating a prey density term could take the form

$$H = y\dot{M}_{scope}\left(\frac{\rho}{\rho_{max}}\right) + b \tag{2.5-1}$$

where y and b are fitting parameters, $\dot{M}_{scope}$ sets the potential (maximum) habitat preference, and the normalized prey density ρ/ρ_{max} is a scaling factor to obtain the realized preference H. A similar prey-availability index ranging from 0 to 1 is used in the SEAPODYM ecosystem model (Lehodey et al., 2008). In non-extrapolative applications (e.g. fisheries monitoring), this prey-availability term could be allowed to vary over space to estimate a time-varying abundance term. For example, the commonly estimated abundance effect (I_t) in fisheries models could be derived using an equation of the form (modified from Maunder et al. (2006))

$$I_t = \frac{C_{n,t}}{H_{n,t}E_{n,t}} \tag{2.5-2}$$

where $C_{n,t}$ is the observed catch at location *n* and time *t*, $H_{n,t}$ is the estimated habitat preference according to equation (2.5-1) (with y and b as constants, $\dot{M}_{scope}$ allowed to vary over time and space and $\frac{\rho}{\rho_{max}}$ only over space) and $E_{n,t}$ is the observed fishing effort (e.g. number of hooks).

Conceptually, the methodology we present for linking the habitat preference H to species distribution is similar to the statistical habitat-based standardization method (statHBS) described by Maunder et al. (2006). In both cases, a function that takes environmental parameters as inputs and returns the estimated species distribution is assumed to take a certain form (our study assumes a substantially more complicated form for these equations than do Maunder et al.), and then a statistical process is used to fit this function to some dataset of observed species distributions. While in a typical statHBS model the fitting parameters determine the effect of each environmental variable on the species distribution (e.g. Bigelow and Maunder (2007)), in our case the cumulative effect of all the environmental variables is calculated according to our aerobic scope model (driven by fixed physiological input parameters), and the fitting parameters try to match this cumulative effect against the observed distribution. Although the benefit of our method is that it is less liable to over-fitting, this comes at a cost of a reliance on a large volume of physiological data. Especially in complex models incorporating predator-prey interactions between multiple species, resource and data constraints will likely dictate that only a few focal species are parameterized with our aerobic scope model, while other species may need to rely on simpler CEM-type models.

2.5.4. Predicting the outcome of selection pressures

Hinton and Nakano (1996) described a deterministic habitat-based standardization for blue marlin where input parameters (habitat preferences) were drawn not from a statistical fitting scheme as in statHBS but through studies of electronically tagged fish. By allowing the input parameters to vary according to a multinomial distribution, the authors were able to estimate confidence intervals in their predicted abundance trends. The aerobic scope model we describe also allows us to use this procedure with the added benefit that the nature of our input variables allows to us decompose the variability in estimated aerobic scope between the physiological and environmental input parameters. The variability driven by the physiological input parameters is particularly interesting because it provides an estimate of how selective pressures acting to modify the frequency of phenotypes already present in wild populations could mitigate any fitness decrement expected under future climate change. For example, our analysis of *T. albacares* in the Eastern Tropical Pacific suggests that these selective pressures could mitigate some, but not all of the projected habitat compression resulting from climate change. Specifically, at some depths such as the upper thermocline, these selective processes may reduce the effects of climate change-induced vertical habitat compression by as much as 50%, while climate change effects are likely to be unaffected by adaptive compensation at deeper depths. Increased data collection (e.g. more physiological measurements from *T. albacares* in the same or from different populations) could be used to further constrain the distribution of physiological phenotypes in the wild and improve the confidence in these predictions.

Another perspective on this problem is provided by the global sensitivity analysis. One interpretation of the sensitivity indices presented in Figure 8 is that parameters assigned large sensitivity values (such as the percent oxygen utilization $O_{utilization}$) have a large impact on the estimated aerobic scope, and therefore more accurate measurements of these variables could contribute most to a narrowing of the confidence bounds of the model. A parallel interpretation that is relevant to the adaptation problem is that high sensitivity indices show the parameters that are most likely to respond to selective pressures because changes in these parameters (either through selective enrichment of more extreme phenotypes or through genetic/epigenetic evolution) can result in substantial changes in aerobic scope.

It is important to note however that our analysis assumes that all of the physiological input parameters are independent, and therefore fails to take into account some adaptive strategies that would necessitate modifications to multiple input parameters. For example, the large total sensitivity index for $O_{utilization}$ (defined as the ratio of pO_2 in the arterial bloodstream verses the pO_2 in the inspired water) indicates that all else being equal, increasing the arterial pO_2 could contribute substantially to maintaining a high aerobic scope. One of the primary methods for increasing arterial pO_2 in fishes is by increasing the residence time of arterial blood in the gills, which can be accomplished most easily by decreasing cardiac output. Cardiac output however is directly proportional to aerobic scope via the Fick equation (2.3-12), therefore increasing $O_{utilization}$, if accomplished via a decline in cardiac output, is likely to be counterproductive in this case. On the other hand, $O_{utilization}$ can also be boosted without affecting cardiac output by modifying the architecture of the gills (e.g. by increasing lamellar surface area, (Sollid et al., 2003)), and such adaptations are much more likely to produce an effective response to future climate change.

2.5.5. Applicability to other species

While we have presented only two parameterizations of our model (the generic teleost model using input parameters from Table 3 and the *T. albacares* model using the parameters from Table 4), our model was deliberately constructed to be compatible with a wide range of marine teleosts. In the case of well-studied species including *Gadus morhua* (Atlantic cod), *Oreochromis mossambicus* (Mozambique tilapia), *Mugil cephalus* (striped mullet) and *Katsuwonus pelamis* (skipjack tuna), most if not all of the input parameters necessary for this model can be gleaned from published laboratory experiments. For other less-studied species, the necessity for data collection can be mitigated somewhat by utilizing available information for congeners or other relatives in the case of variables to which the model has low sensitivity, such as the hill number or arterial pH (Figure 8, variables pH_a and η). Predicting the range shifts of some species such as *T. albacares* and *G. morhua* is of particular interest because studies have already shown significant changes in distribution due either to ongoing climate change (Rose, 2004) or episodic climate events such as the El Niño Southern Oscillation (Lehodey et al., 1997; Pearcy, 2002). Integrating our habitat suitability formulation as a single-species add-on to end-to-end ecosystem models such as SEAPODYM, APECOSM or ATLANTIS (Lehodey et al., 2008; Link et al., 2010; Maury, 2010) could allow researchers to improve their analysis of how climate change perturbations in the distribution of these species might interact with food web structure and biogeochemical cycling. We have also described in section 2.5.3 how fisheries assessment models could integrate our habitat suitability index. We anticipate that both these cases, the physiological basis of our model and the resulting robustness to extrapolation and over fitting will improve the quality of these efforts.

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2.7. References

Berenbrink, M., Bridges, C.R., 1994. Catecholamine-Activated Sodium/Proton Exchange in the Red Blood Cells of the Marine Teleost Gadus Morhua. J. Exp. Biol. 192, 253–67.

Bernier, N.J., Fuentes, J., Randall, D.J., 1996. Adenosine receptor blockade and hypoxia-tolerance in rainbow trout and pacific hagfish. J. Exp. Biol. 199, 497–507.

Bigelow, K. a, Maunder, M.N., 2007. Does habitat or depth influence catch rates of pelagic species? Can. J. Fish. Aquat. Sci. 64, 1581–1594. doi:10.1139/f07-115

Bijma, J., Pörtner, H.-O., Yesson, C., Rogers, A.D., 2013. Climate change and the oceans--what does the future hold? Mar. Pollut. Bull. 74, 495–505. doi:10.1016/j.marpolbul.2013.07.022

Blank, J.M., Morrissette, J.M., Davie, P.S., Block, B. a, 2002. Effects of temperature, epinephrine and Ca(2+) on the hearts of yellowfin tuna (Thunnus albacares). J. Exp. Biol. 205, 1881–8.

Braaten, P.J., Dey, P.D., Annear, T.C., 1997. Development and evaluation of bioenergetic-based habitat suitability criteria for trout. Regul. Rivers Res. Manag. 13, 345–356.

Brauner, C.J., Randall, D.J., 1998. The linkage between oxygen and carbon dioxide transport, in: Perry, S.F., Bruce, L. (Eds.), Fish Physiology, Volume 17: Fish Respiration. Academic Press, pp. 283–319.

Brewer, P.G., Peltzer, E.T., 2009. Limits to Marine Life. Science (80-.). 324, 347–348.

Brill, R.W., Block, B.A., Boggs, C.H., Bigelow, K.A., Freund, E. V, Marcinek, D.J., 1999. Horizontal movements and depth distribution of large adult yellowfin tuna (Thunnus albacares) near the Hawaiian Islands, recorded using ultrasonic telemetry : implications for the physiological ecology of pelagic fishes. Mar.

Biol. 133, 395–408. doi:10.1007/s002270050478

Brill, R.W., Bushnell, P.G., 1991. Effects of open- and closed-system temperature changes on blood oxygen dissociation curves of skipjack tuna, Katsuwonus pelamis, and yellowfin tuna, Thunnus. Can. J. Zool. 69, 1814–1821.

Burton, R.F., 1996. The dependence of normal arterial blood pH on sodium concentration in teleost fish. Comp. Biochem. Physiol. Part A Physiol. 114, 111–116. doi:10.1016/0300-9629(95)02102-7

Bushnell, P.G., 1988. Cardiovascular and respiratory responses to hypoxia in three species of obligate ram ventilating fishes, skipjack tuna (Katsuwonus pelamis), yellowfin tuna (Thunnus albacares), and bigeye tuna (T. obesus). University of Hawaii.

Bushnell, P.G., Brill, R.W., 1991. Responses of Swimming Skipjack (Katsuwonus pelamis) and Yellowfin (Thunnus albacares) Tunas to Acute Hypoxia, and a Model of Their Cardiorespiratory Function. Physiol. Zool. 64, 787–811.

Cai, W.-J., Hu, X., Huang, W.-J., Murrell, M.C., Lehrter, J.C., Lohrenz, S.E., Chou, W.-C., Zhai, W., Hollibaugh, J.T., Wang, Y., Zhao, P., Guo, X., Gundersen, K., Dai, M., Gong, G.-C., 2011. Acidification of subsurface coastal waters enhanced by eutrophication. Nat. Geosci. 4, 766–770. doi:10.1038/ngeo1297

Cameron, B.Y.J.N., Polhemus, J.A., 1974. Theory of CO2 exchage in trout gills. J. Exp. Biol. 60, 183–194.

Chung, W.-S., Marshall, N.J., Watson, S.-A., Munday, P.L., Nilsson, G.E., 2014. Ocean acidification slows retinal function in a damselfish through interference with GABAA receptors. J. Exp. Biol. 217, 323–6. doi:10.1242/jeb.092478

Claireaux, G., Lagardére, J.P., 1999. Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. J. Sea Res. 42, 157–168. doi:10.1016/S1385-1101(99)00019-2

Crain, C.M., Kroeker, K., Halpern, B.S., 2008. Interactive and cumulative effects of multiple human stressors in marine systems. Ecol. Lett. 11, 1304–15. doi:10.1111/j.1461-0248.2008.01253.x

Dagorn, L., Holland, K.N., Hallier, J., Taquet, M., Moreno, G., Sancho, G., Itano, D.G., Aumeeruddy, R., Girard, C., Million, J., Fonteneau, A., 2006. Deep diving behavior observed in yellowfin tuna (Thunnus albacares). Aquat. Living Resour. 88, 85–88. doi:10.1051/alr

Decker, H., Nadja, H., 2007. Negative cooperativity in Root-effect hemoglobins: role of heterogeneity. Integr. Comp. Biol. 47, 656–61. doi:10.1093/icb/icm073

Denny, M., Helmuth, B., 2009. Confronting the physiological bottleneck: A challenge from ecomechanics. Integr. Comp. Biol. 49, 197–201. doi:10.1093/icb/icp070

Dewar, H., Graham, J.B., 1994. Studies of Tropical Tuna Swimming Performance in a Large Water Tunnel - Energetics. J. Exp. Biol. 192, 45–59.

Dotson, R.C., 1976. Minimum Swimming Speed of Albacore, Thunnus alalunga. Fish. Bull. 74, 955–960.

Essington, T., Schindler, D., Olson, R.J., Kitchell, J.F., Boggs, C., Hilborn, R., 2002. Alternative fisheries and the predation rate of yellowfin tuna in the eastern pacific ocean. Ecol. Appl. 12, 724–734.

Evans, J.J., Shoemaker, C. a, Klesius, P.H., 2003. Effects of sublethal dissolved oxygen stress on blood glucose and susceptibility to Streptococcus agalactiae in

Nile tilapia Oreochromis niloticus. J. Aquat. Anim. Health 15, 202–208. doi:10.1577/H03-024

Farrell, A.P., 1996. Features Heightening Cardiovascular Performance in Fishes , with Special Reference to Tunas. Comp. Biochem. Physiol. Part A 113, 61–67.

Farrell, A.P., 2002. Cardiorespiratory performance in salmonids during exercise at high temperature: insights into cardiovascular design limitations in fishes. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 132, 797–810.

Filho, D.W., Eble, G.J., Kassner, G., Caprario, F.X., Dafré, A.L., Ohira, M., 1992. Comparative hemotology in marine fish. Comp. Biochem. Physiol. Part A 102, 311–321.

Forster, M.E., Axelsson, M., Farrell, A.P., Nilsson, S., 1991. Cardiac function and circulation in hagfishes. Can. J. Fish. Aquat. Sci. 69, 1985–1992.

Fry, F.E.J., 1947. Effects of the environment on animal activity. Publ. Ontario Fish. Res. Lab. 55, 1–62.

Gaesser, G., Brooks, G., 1983. Metabolic bases of excess post-exercise oxygen consumption: a review. Med. Sci. Sports Exerc. 16, 29–43.

Garcia, H.E., Locarnini, R.A., Boyer, T.P., Antonov, J.I., Baranova, O.K., Zweng, M.M., Reagan, J.R., Johnson, D.R., 2014. World Ocean Atlas 2013, Volume 3: Dissolved oxygen, apparent oxygen utilization, and oxygen saturation.

Gruber, N., 2011a. Warming up, turning sour, losing breath: ocean biogeochemistry under global change. Philos. Trans. R. Soc. A 369, 1980–1996. doi:10.1098/rsta.2011.0003

Gruber, N., 2011b. Warming up, turning sour, losing breath: ocean biogeochemistry under global change. Philos. Trans. A. Math. Phys. Eng. Sci. 369, 1980–96. doi:10.1098/rsta.2011.0003

Herman, J., Woodruff, M., Mutel, C., Rios, F., Hyams, D., 2014. SALib.

Hijmans, R.J., Graham, C.H., 2006. The ability of climate envelope models to predict the effect of climate change on species distributions. Glob. Chang. Biol. 12, 2272–2281. doi:10.1111/j.1365-2486.2006.01256.x

Hill, A. V, 1910. The possible effects of the aggregation of the molecules of hemoglobin on its dissociation curves. Proc. Physiol. Soc. 4–7.

Hinton, M.G., Nakano, H., 1996. Standardizing catch and effort statistics using physiological, ecological, or behavioral constraints, and environmental data, with applications to blue marlin (Makaira nigricans) and swordfish (Xiphias gladius) of the Pacific Ocean. University of California, San Diego.

Hirzel, A.H., Le Lay, G., 2008. Habitat suitability modelling and niche theory. J. Appl. Ecol. 45, 1372–1381. doi:10.1111/j.1365-2664.2008.01524.x

Homyack, J. a., 2010. Evaluating habitat quality of vertebrates using conservation physiology tools. Wildl. Res. 37, 332. doi:10.1071/WR08093

Hutchinson, G.E., 1957. Concluding remarks, in: Cold Spring Harbor Symposia on Quantitative BIology Vol. 22. Cold Spring Harbor Laboratory Press.

Ishimatsu, A., Hayashi, M., Lee, K., 2005. Physiological effects on fishes in a high-CO2 world. J. Geophys. Res. 110, 1–8. doi:10.1029/2004JC002564

Ishimatsu, A., Kikkawa, T., Hayashi, M., Lee, K.-S., Kita, J., 2004. Effects of CO2 on Marine Fish: Larvae and Adults. J. Oceanogr. 60, 731–741.

Jensen, F.B., 2004. Red blood cell pH, the Bohr effect, and other oxygenation-linked phenomena in blood O2 and CO2 transport. Acta Physiol. Scand. 182, 215–27. doi:10.1111/j.1365-201X.2004.01361.x

Kato, T., Tsukanaka, A., Harada, T., Kosaka, M., Matsui, N., 2005. Effect of hypercapnia on changes in blood pH, plasma lactate and ammonia due to exercise. Eur. J. Appl. Physiol. 95, 400–8. doi:10.1007/s00421-005-0046-z

Kearney, M., Porter, W., 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. Ecol. Lett. 12, 1–17.

Key, R.M., Kozyr, A., Sabine, C.L., Lee, K., Wanninkhof, R., Bullister, J., Feely, R.A., Millero, F., Mordy, C., Peng, T.-H., 2004. A global ocean carbon climatology: Results from GLODAP. Global Biogeochem. Cycles 18, 1–23.

Korsmeyer, K.E., Lai, N.C., Shadwick, R.E., Graham, J.B., 1997. Oxygen transport and cardiovascular responses to exercise in the yellowfin tuna Thunnus albacares. J. Exp. Biol. 200, 1987–1997.

Kunzmann, A., 1991. Blood physiology and ecological consequences in Weddell Sea fishes. University of Kiel.

Kutty, M.N., 1968. Respiratory quotients in goldfish and rainbow trout. J. Fish. Board Canada 25, 1689–1728.

Lavigne, H., Epitalon, J.-M., Gattuso, J.-P., 2014. seacarb: seawater carbonate chemistry with R.

Lee, C.G., Devlin, R.H., Farrell, A.P., 2003. Swimming performance, oxygen consumption and excess post-exercise oxygen consumption in adult transgenic and ocean-ranched coho salmon. J. Fish Biol. 62, 753–766. doi:10.1046/j.0022-1112.2003.00057.x

Lee, K.-S., Kita, J., Ishimatsu, A., 2003. Effects of lethal levels of environmental hypercapnia on cardiovascular and blood-gas status in yellowtail, Seriola quinqueradiata. Zoolog. Sci. 20, 417–22.

Lefrançois, C., Claireaux, G., 2003. Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole Solea solea. Mar. Ecol. Prog. Ser. 259, 273–284.

Lehodey, P., Bertignac, M., Hampton, J., Lewis, A., Picaut, J., 1997. El Niño Southern Oscillation and tuna in the western Pacific. Nature 389, 715–718.

Lehodey, P., Senina, I., Murtugudde, R., 2008. A spatial ecosystem and populations dynamics model (SEAPODYM) – Modeling of tuna and tuna-like populations. Prog. Oceanogr. 78, 304–318. doi:10.1016/j.pocean.2008.06.004

Lehodey, P., Senina, I., Sibert, J., Bopp, L., Calmettes, B., Hampton, J., Murtugudde, R., 2010. Preliminary forecasts of Pacific bigeye tuna population trends under the A2 IPCC scenario. Prog. Oceanogr. 86, 302–315. doi:10.1016/j.pocean.2010.04.021

Link, J.S., Fulton, E.A., Gamble, R.J., 2010. The northeast US application of ATLANTIS: A full system model exploring marine ecosystem dynamics in a living marine resource management context. Prog. Oceanogr. 87, 214–234. doi:10.1016/j.pocean.2010.09.020

Link, J.S., Gamble, R.J., Fogarty, M.J., 2011. An Overview of the NEFSC's Ecosystem Modeling Enterprise for the Northeast US Shelf Large Marine Ecosystem: Towards Ecosystem-based Fisheries Management. Woods Hole, MA.

Locarnini, R.A., Mishonov, A. V, Antonov, J.I., Boyer, T.P., Garcia, H.E., Baranova, O.K., Zweng, M.M., Paver, C.R., Reagan, J.R., Johnson, D.R., Hamilton, M., Seidov, D., 2013. World Ocean Atlas 2013, Volume 1: Temperature.

Lowe, T.E., Brill, R.W., 1998. Responses of the red blood cells from two high-energy-demand teleosts, yellowfin tuna (Thunnus albacares) and skipjack tuna (Katsuwonus pelamis), to catecholamines. J. Comp. Physiol. B 168, 405–418.

Magnuson, J.J., 1973. Comparative Study of Adaptations for Continuous Swimming and Hydrostatic Equilibrium of Scombroid and Xiphoid Fishes. Fish. Bull. 71, 337–356.

Maunder, M.N., Hinton, M.G., Bigelow, K.A., Langley, A.D., 2006. Developing Indices of Abundance Using Habitat Data in a Statistical Framework. Bull. Mar. Sci. 79, 545–559.

Maury, O., 2010. An overview of APECOSM, a spatialized mass balanced “Apex Predators ECOSystem Model” to study physiologically structured tuna population dynamics in their ecosystem. Prog. Oceanogr. 84, 113–117.

Melzner, F., Gutowska, M.A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M.C., Bleich, M., 2009. Physiological basis for high CO2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosciences 6, 2313–2331.

Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. a., Bange, H.W., Hansen, H.P., Körtzinger, A., 2012. Future ocean acidification will be amplified by hypoxia in coastal habitats. Mar. Biol. 160, 1875–1888. doi:10.1007/s00227-012-1954-1

Nagarajan, R., 2002. Quantifying physiological data with Lempel-Ziv complexity - certain issues. IEEE Trans. Biomed. Eng. 49, 1371–3. doi:10.1109/TBME.2002.804582

Nilsson, G.E., Dixson, D.L., Domenici, P., McCormick, M.I., Sørensen, C., Watson, S., Munday, P.L., 2012. Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. Nat. Clim. Chang. 1–4. doi:10.1038/NCLIMATE1352

Pearcy, W.G., 2002. Marine nekton off Oregon and the 1997–98 El Nino. Prog. Oceanogr. 54, 399–403.

Perry, S.F., Gilmour, K.M., 1996. Consequences of catecholamine release on ventilation and blood oxygen transport during hypoxia and hypercapnia in an elasmobranch Squalus acanthias and a teleost Oncorhynchus mykiss. J. Exp. Biol. 199, 2105–18.

Pörtner, H.-O., 2002. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 132, 739–61.

Pörtner, H.-O., 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. J. Exp. Biol. 213, 881–93. doi:10.1242/jeb.037523

Powers, A., 1980. Molecular Ecology of Teleost Fish Hemoglobins: Strategies for Adapting to Changing Environments. Am. Zool. 20, 139–162.

Prince, E.D., Goodyear, C.P., 2006. Hypoxia-based habitat compression of tropical pelagic fishes. Fish. Oceanogr. 15, 451–464. doi:10.1111/j.1365-

2419.2005.00393.x

Richardson, H.B., 1923. The respiratory quotient. Physiol. Rev. 9, 61–125.

Roch, M., Maly, E., 1979. Relationship of cadmium-induced hypocalcemia with mortality in rainbow trout (Salmo gairdneri) and the influence of temperature on toxicity. J. Fish. Board Canada 36, 1297–1303.

Rodolfo-Metalpa, R., Houlbrèque, F., Tambutté, É., Boisson, F., Baggini, C., Patti, F.P., Jeffree, R., Fine, M., Foggo, a., Gattuso, J.-P., Hall-Spencer, J.M., 2011. Coral and mollusc resistance to ocean acidification adversely affected by warming. Nat. Clim. Chang. 1, 308–312. doi:10.1038/nclimate1200

Rosa, R., Seibel, B. a, 2008. Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. Proc. Natl. Acad. Sci. U. S. A. 105, 20776–80. doi:10.1073/pnas.0806886105

Rose, G.A., 2004. Reconciling overfishing and climate change with stock dynamics of Atlantic cod (Gadus morhua) over 500 years. Can. J. Fish. Aquat. Sci. 61, 1553–1557. doi:10.1139/F04-173

Rosenfeld, J.S., Leiter, T., Lindner, G., Rothman, L., 2005. Food abundance and fish density alters habitat selection, growth, and habitat suitability curves for juvenile coho salmon (Oncorhynchus kisutch). Can. J. Fish. Aquat. Sci. 62, 1691–1701. doi:10.1139/f05-072

Saltelli, A., Annoni, P., Azzini, I., Campolongo, F., Ratto, M., Tarantola, S., 2010. Variance based sensitivity analysis of model output. Design and estimator for the total sensitivity index. Comput. Phys. Commun. 181, 259–270. doi:10.1016/j.cpc.2009.09.018

Schaefer, K.M., Fuller, D.W., 2004. Behavior of bigeye (Thunnus obesus) and skipjack (Katsuwonus pelamis) tunas within aggregations associated with floating objects in the equatorial eastern Pacific. Mar. Biol. 146, 781–792. doi:10.1007/s00227-004-1480-x

Schaefer, K.M., Fuller, D.W., Block, B.A., 2007. Movements, behavior, and habitat utilization of yellowfin tuna (Thunnus albacares) in the northeastern Pacific Ocean, ascertained through archival tag data. Mar. Biol. 152, 503–525. doi:10.1007/s00227-007-0689-x

Schaefer, K.M., Fuller, D.W., Block, B.A., 2009. Vertical Movements and Habitat Utilization of Skipjack (Katsuwonus pelamis), Yellowfin (Thunnus albacares), and Bigeye (Thunnus obesus) Tunas in the Equatorial Eastern Pacific Ocean, Ascertained Through Archival Tag Data, in: Nielsen, J.L., Arrizabalaga, H., Fagoso, N., Hobday, A., Lutcavage, M., Sibert, J. (Eds.), Reviews: Methods and Technologies in Fish Biology and Fisheries.Tagging and Tracking of Marine Animals with Electronic Devices. Springer Dordrecth Heidelberg, New York, pp. 121–144.

Schmittner, A., Oschlies, A., Matthews, H.D., Galbraith, E.D., 2008. Future changes in climate, ocean circulation, ecosystems, and biogeochemical cycling simulated for a business-as-usual CO 2 emission scenario until year 4000 AD. Global Biogeochem. Cycles 22, 1–21. doi:10.1029/2007GB002953

Selzer, A., Sudrann, R.B., 1958. Reliability of the Determination of Cardiac Output in Man by Means of the Fick Principle. Circ. Res. 6, 485–490. doi:10.1161/01.RES.6.4.485

Siggaard-Andersen, O., Garby, L., 1973. The Bohr effect and the Haldane effect.

Scandanavian J. fof Clin. Lab. Investig. 31, 1–8.

Thannickal, V.J., 2009. Oxygen in the evolution of complex life and the price we pay. Am. J. Respir. Cell Mol. Biol. 40, 507–10. doi:10.1165/rcmb.2008-0360PS

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., De Siqueira, M.F., Grainger, A., Hannah, L., Hughes, L., Huntley, B., Van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M. a, Peterson, a T., Phillips, O.L., Williams, S.E., 2004. Extinction risk from climate change. Nature 427, 145–8. doi:10.1038/nature02121

Toews, B.Y.D.P., Holetonj, G.F., Heisler, N., 1983. Regulation of the Acid-Base Status During Environmental Hypercapnia in the Marine Teleost Fish Conger conger. J. Exp. Biol. 107, 9–20.

Travis, J.M.J., 2003. Climate change and habitat destruction: a deadly anthropogenic cocktail. Proc. Biol. Sci. 270, 467–73. doi:10.1098/rspb.2002.2246

Wood, C.M., McMahon, B.R., McDonald, D.G., 1979. Respiratory gas exchange in the resting starry founder, Platichthys stellatus: A comparison with other teleosts. J. Exp. Biol. 78, 167–179.

Yukimoto, S., Yoshimura, H., Hosaka, M., Sakami, T., Tsujino, H., Hirabara, M., Tanaka, T.Y., Deushi, M., Obata, A., Nakano, H., Adachi, Y., Shindo, E., Yabu, S., Ose, T., Kitoh, A., 2011. Meteorological Research Institute Earth System Model Version 1 (MRI-ESM1): Model Description. Tech. Reports Meteorol. Res. Inst. 64, 88.

2.8. Figures and Tables:

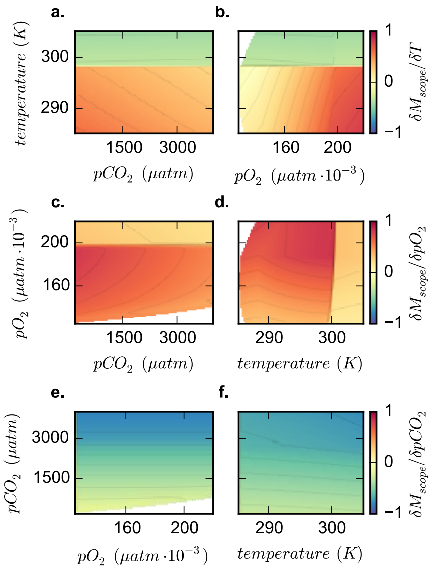

Figure 4: Aerobic scope model reveals complex putative interactions of environmental variables in a generic teleost fish. (**a**) Change in estimated $\dot{M}_{scope}$ with increasing temperature (normalized to the range 0-1 and averaged across all possible pO_2 values) is shown for all combinations of temperature and pCO_2. Colors indicate regions where $\dot{M}_{scope}$ increases (red) or decreases (blue) with increasing temperature. Contours (black lines) help to visualize patterns in the effect size. The slant in the contour lines below temperatures of 298 K (the value of the cardiac temperature threshold T_T, see Table 3) for example reveals that increasing temperature has the largest effect on $\dot{M}_{scope}$ ($\partial\dot{M}_{scope}/\partial T$ is highest) at low temperatures and low pCO_2. (**b**) $\partial\dot{M}_{scope}/\partial T$ across the temperature/pO_2 space, $\partial\dot{M}_{scope}/\partial pO_2$ across the (**c**) pO_2/pCO_2 and (**d**) pO_2/temperature space, and $\partial\dot{M}_{scope}/\partial pCO_2$ across the (**e**) pCO_2/pO_2 and (**f**) pCO_2/temperature space. Physiological parameters for this model realization are shown in Table 3 with typical values from the literature.

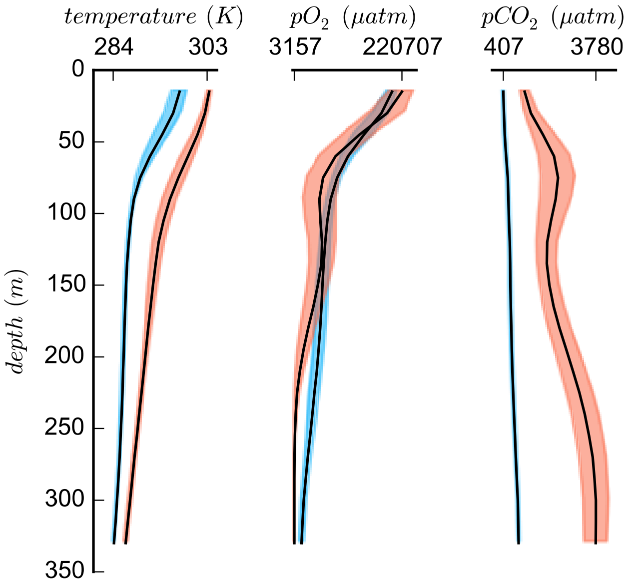

Figure 5: Projected multivariate perturbations in environmental variables for the study region in the Eastern Tropical Pacific oxygen minimum zone. Depth profiles of mean (black line) and ± 1 standard deviation (shaded) temperature, pO_2 and pCO_2 for the latitudes 5°S to 5°N and longitudes 90°W to 105°W. Current conditions (blue) are drawn from the World Ocean Atlas 2013 (temperature, pO_2) and the Global Ocean Data Analysis Project (pCO_2). Projected end-century conditions (orange) are drawn from the Meteorological Research Institute-Earth System Model Version 1 (MRI-ESM1) for the year 2100 under the RCP 8.5 scenario.

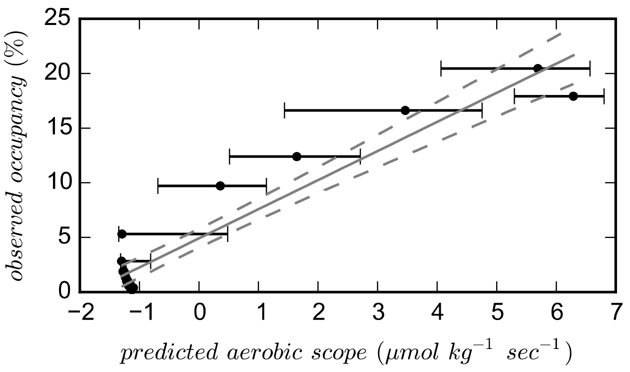

Figure 6: Estimated aerobic scope strongly predicts the observed distribution of *T. albacares*. The vertical distribution of *T. albacares* (% time spent at depth, bin size 15m) tracked by electronic tags in the study region (y axis, Schaefer et al., 2007) shows a strong correlation with aerobic scope (x axis) estimated by the model in the same region using environmental parameters drawn from the World Ocean Atlas 2013 and the Global Ocean Data Analysis Project. Error bars show the interquartile range of estimated aerobic scope (some error bars are too small to see). The grey line and dotted curves show the mean and 95% confidence bounds of a linear regression (N = 22 observations, $R^2 = 0.91$, $p < 0.0001$).

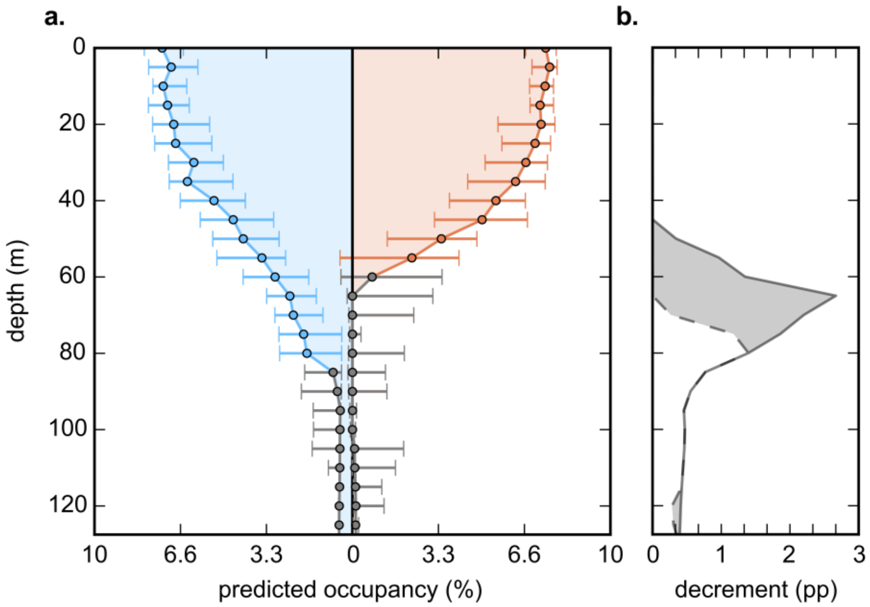

Figure 7: Climate-change driven reduction in vertical habitat for *T. albacares*. (**a**) Using the linear regression model shown in Figure 6, we predict the vertical habitat of *T. albacares* under the current (blue) and end-century (orange) conditions shown in Figure 5. Error bars show the interquartile range of estimated occupancy at a given depth obtained using a bootstrap sampling procedure of environmental and physiological variables (n = 1000). Grey dots in both the current and future

projections show depths where estimated aerobic scope is below zero, indicating these depths are accessible only through temporary forays (such as for foraging or thermoregulation) and not long-term habitation. (**b**) The median decrement in occupancy between the current and future scenarios (solid line) is compared against the decrement (in percentage points) at the 5th percentile (dotted line) when estimates are obtained by bootstrap sampling of physiological parameters from their observed distributions (n = 1000). The 5th percentile was chosen in this figure as a plausible value for the upper range of adaptive capacity. The distance between the median and 5th percentile decrement (grey shaded area) indicates the ability of currently existing physiological plasticity to mitigate changes in vertical habitat caused by climate change.

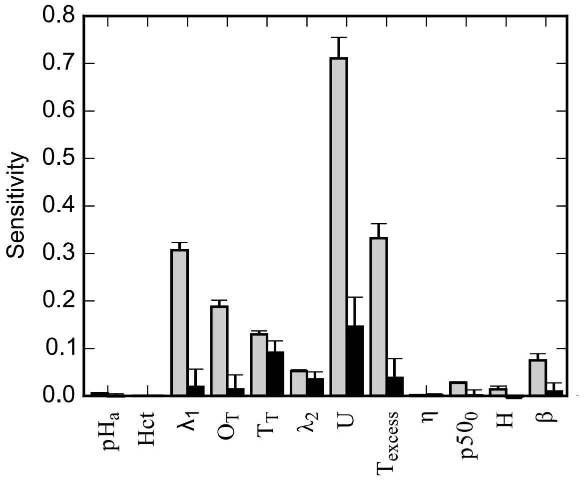

Figure 8: Sensitivity of estimated aerobic scope to input parameters. Bars show magnitude of Monte Carlo estimations of Sobol sensitivity indices. First order main effects (black bars) indicate the effect of uncertainty in the given parameter on the uncertainty of the estimated aerobic scope. Total effects (grey bars) show the sum of the main effects plus interactions between the given parameter and all other

parameters. Error bars indicate one standard deviation. Parameter labels correspond to those used in Table 3.

Table 3: List of input parameters for the generic marine teleost model

Parameter (units)	Symbol	Typical value (range)	Reference
Blood Parameters			
Extracellular Bohr Coefficient (Δlogp50/ΔpH)	β	-1.1 (-1.4, -0.2)	Brauner and Randall, 1998
Heat of Oxygenation (kcal/mole)	H	-15 (-17, -1)	Powers, 1980
Hemoglobin half-saturation constant (μatm)	$p50$	15000 (2400, 26800)^2	Bernier et al., 1996; Korsmeyer et al., 1997
Reference pH	pH_0	7.8	N/A
Reference Temperature (K)	T_0	293.15	N/A
Hemoglobin Oxygen Capacity (mol O_2/g Hb)	O_H	6.95×10^{-5}	Korsmeyer et al., 1997
Mean Cell Hemoglobin Concentration (g Hb/L)	$\bar{H}$	212 (125-381)^1	Filho et al., 1992
Hematocrit (volume fraction)	Hct	0.41 (0.12, 0.76)^1	Filho et al., 1992
Arterial pH	pH_a	7.8 (7.74, 8.08)	Burton, 1996
Oxygen Utilization (partial pressure fraction)	U	0.7 (0.25, 0.85)	Wood et al., 1979
Hill Coefficient	η	1.5 (0.6, 2.0)	Decker and Nadja, 2007
Minimum venous pO_2 (μatm)	pO_{2v}	75000 (50000, 100000)^3	Korsmeyer et al., 1997; Kunzmann, 1991

Cardiac Parameters			
O_2 Threshold (mol O_2/L blood)	O_T	0.00475 ±0.0005	Bushnell, 1988
O_2 Slope (L^2 blood/ kg body sec mol O_2)	λ_1	330 (0, 340)	Bushnell, 1988
Temperature Threshold (K)	T_T	298.15 (276.15, >298.15)[3]	Blank et al., 2002
Temperature Slope (L blood/kg body sec K)	λ_2	0.044 (0, 0.05)	Blank et al., 2002
Reference Cardiac Output (L blood /kg body sec)	Q_{max}	0.001 (0.0004, 0.00196)[2]	Forster et al., 1991; Korsmeyer et al., 1997
Coronary oxygen supply (fraction)	f	0.3 (0, 1.0)	Farrell, 1996
Excess Muscle Temperature (K)	T_{excess}	0 (0, 20)	Brill et al., 1999

N/A: not applicable

[1] Excludes white-blooded Antarctic Notothenioids (*Channichthyidae*) that lack hemoglobin or red blood cells ($\bar{H}, Hct = 0$)

[2] Range constructed using hagfish and tunas as endpoints

[3] Range constructed using white-blooded Antarctic Notothenioids (*Channichthyidae*) and tunas as endpoints

Table 4: List of input parameters for *Thunnus albacares* model

Parameter (units)	Symbol	Values (mean ± SD)	Reference

	Blood Parameters		
Extracellular Bohr Coefficient (ΔlogP50/ΔpH)	β	-0.87 ±0.15	Bushnell, 1988
Heat of Oxygenation (kcal/mole)	H	-1.0 ±2.0	Bushnell, 1988
Hemoglobin half-saturation constant (µatm)	$p50$	26800 ±1320	Korsmeyer et al., 1997
Reference pH	pH_0	7.893	Korsmeyer et al., 1997
Reference Temperature (K)	T_0	293.15	Korsmeyer et al., 1997
Hemoglobin Oxygen Capacity (mol O_2/g Hb)	O_H	6.95×10^{-5}	Korsmeyer et al., 1997
Mean Cell Hemoglobin Concentration (g Hb/L)	$\bar{H}$	370	Korsmeyer et al., 1997
Hematocrit (volume fraction)	Hct	0.283 ±0.01	Korsmeyer et al., 1997
Arterial pH	pH_a	7.73 ±0.01	Korsmeyer et al., 1997
Oxygen Utilization (partial pressure fraction)	U	0.45 ±0.06	Korsmeyer et al., 1997
Hill Coefficient	η	1.72 ±0.06	Bushnell, 1988
Minimum venous pO_2 (µatm)	pO_{2v}	50000	Korsmeyer et al., 1997
	Cardiac Parameters		
O_2 Threshold (µatm)	O_T	0.00475 ±0.0005	Bushnell, 1988

O_2 Slope (L blood/ kg body sec μatm O_2)	λ_1	337.7 ±120.9	Bushnell, 1988
Temperature Threshold (K)	T_T	298.15 ±2.0	Blank et al., 2002
Temperature Slope (L blood/kg body sec K)	λ_2	0.044 ±0.007	Blank et al., 2002
Reference Cardiac Output (L blood /kg body sec)	Q_{max}	0.0196	Korsmeyer et al., 1997
Coronary oxygen supply (fraction)	f	1.0	Farrell, 1996
Excess Muscle Temperature (K)	T_{excess}	5.0 ±3.0	Brill et al., 1999

*Values given without standard deviations were treated as constants

3. Synergistic Impact of Simultaneous Climate-Related Parameters on Aerobic Scope in a Model Teleost, *Oreochromis mossambicus*

3.1. Abstract

Multiple-stressor perturbations, where simultaneous environmental changes act in concert to disrupt physiological functioning, will characterize much of the impacts of climate change on marine teleosts. Three stressors that are expected to become especially prevalent by the end of the century are CO_2-induced ocean acidification, surface-ocean warming, and deoxygenation. Using the euryhaline Mozambique tilapia (*Oreochromis mossambicus*) as a model teleost, we explore the effects of these stressors both in isolation and in combination on aerobic scope, a key physiological indicator of organism fitness, and blood-based stress indicators including glucose, base excess, pCO_2, and venous oxygen saturation. Our results show that while neither increased temperature nor pCO_2 alone are sufficient to cause substantial declines in the aerobic scope of *O. mossambicus*, these same stressors have a significant effect when combined with simultaneous deoxygenation. A combination of stressors was also more effective than single stressors at eliciting responses in most blood stress parameters. In particular, both deoxygenation and acidification interact synergistically with warming, suggesting that the total impact of climate-change related stresses may be substantially higher than what is indicated by single-variable studies.

3.2. Introduction

Understanding the cumulative impact of multiple simultaneous stressors on organism functioning and population distribution remains one of the most pressing challenges for conservation biology (Crain et al., 2008). This issue is particularly important in the marine context, where a variety of anthropogenic stressors including pollutants, habitat alteration, and nutrient input affect organisms across large areas of the ocean (Adams, 2005). Whereas it is often assumed that the impact of multiple stressors can be approximated as a simple additive accumulation of the impacts associated with single stressors, recent studies have revealed the prevalence of complex synergistic or compensatory interactions (Crain et al., 2008). Further experimental data on organism responses to simultaneous stressors is necessary to contribute to a more general understanding of these effects.

Anthropogenic climate change will become an increasingly important driver of simultaneous environmental perturbation of the marine environment during this century. Ocean acidification (and attendant high dissolved CO_2), ocean deoxygenation, and surface ocean warming are three of the most important physical changes that are likely to co-occur in much of the ocean (Bopp et al., 2013). All three are known to strongly affect the oxygen transport system of marine teleosts, which in turn can have profound effects on metabolism and fitness (Farrell et al., 2008; Pörtner, 2010; Pörtner et al., 2010). For example, recent studies portend declines in migratory success (Farrell et al., 2008), increased mortality (Munday et al., 2009), and changes in community composition (Nilsson et al., 2009) among various marine teleosts as a result of climate-driven changes in these environmental parameters.

Whereas the theoretical and empirical basis for interactions between temperature and oxygen impacts on water breathing organisms has been fairly well established (Cooke et al., 2008; Lapointe et al., 2014; Pörtner, 2010, 2001), there is much less information available on interactions with hypercapnia (elevated CO_2). Munday et al. (2009) found that although both warming and hypercapnia affect aerobic scope in two species of coral reef fish, interactions between the two stressors were strictly additive. By contrast, Cruz-Neto and Steffensen (1997) revealed increased sensitivity of routine metabolism to hypoxia (low oxygen) in the eel *Anguilla anguilla* when exposed to simultaneous hypercapnia. Similarly, Ginneken et al. (1996) examined the effects of simultaneous hypoxia and acidification (where the pH-perturbation was achieved via acid titration, not CO_2 addition) on the tilapia *Oreochromis mossambicus* and found synergistic effects on intracellular pH and phosphocreatine levels, but not on routine metabolism. Finally, Rosa and Seibel (2008) revealed synergistic interactions between warming, hypoxia, and hypercapnia on routine metabolic rates in the jumbo squid *Dosidicus gigas*. To our knowledge, no study to date has examined interactions between temperature, oxygen, and CO_2 simultaneously on a marine teleost.

An important distinction that deserves discussion here is the disparate impact of pH and pCO_2 on marine organisms. Marine teleosts have a robust ability to regulate arterial pH and are therefore likely to be isolated from the effects of environmental pH perturbations (Ishimatsu et al., 2004; Melzner et al., 2009). One potential exception is that increased bicarbonate uptake, which is required to maintain high arterial pH in an acidified seawater environment, can lead to notable pathologies in teleosts including hypercalcification (Melzner et al., 2012). Furthermore, temporary blood-pH perturbations, if they propagate through the red blood cell membrane, may have a direct effect on blood-oxygen binding due to the binding of free protons (H+) to an allosteric site on the hemoglobin molecule (Jensen, 2004).

By contrast, teleost fishes have a low capacity to ameliorate pCO_2 perturbations. While ocean acidification and environmental hypercapnia often go hand in hand, the ability of the pCO_2 molecule to diffuse across cell membranes means that hypercapnia is often the driving factor for systemic effects. While the specific mechanisms for these effects are not well understood, experimental studies have clearly shown that acidification accompanied by environmental hypercapnia shows substantially more lethality in a variety of teleosts as compared to acidification alone (K.-S. Lee et al., 2003). pCO_2 regulation in teleosts is particularly coarse due to the unavailability of a number of strategies used by other taxa. Elasmobranches, for example, carry carbonic anhydrase (an enzyme that catalyzes the conversion of carbonic acid to aqueous CO_2) in the blood plasma to allow CO_2 produced during metabolic processes to be offloaded efficiently during passage through the gills. Teleosts, by contrast, only carry carbonic anhydrase in the red blood cell, with the result that the rate of CO_2 offloading is primarily limited by diffusion through the red blood cell membrane (Evans et al., 2003). Similarly, while air-breathers are able to raise ventilatory rates to boost CO_2 loss through the gills, high ventilatory rates in water-breathers are limited by the viscosity of the medium (Toews et al., 1983). The net result is that even as arterial pH typically remains stable after an adaptive period of 12-24 hours even under extreme pH perturbation, pCO_2 homeostasis is never achieved in a hypercapnic environment (Ishimatsu et al., 2004). This prolonged pCO_2 perturbation can lead not only to metabolic pathologies but also to a wide range of potential issues including neuronal dysfunction (Nilsson et al., 2012).

Our study examines the impact of temperature, oxygen, and pCO_2 perturbations reflective of the upper range of those expected under end-century climate change on the Mozambique tilapia, *Oreochromis mossambicus*. This species has a native range that is limited to the eastward flowing rivers of central and southern Africa (Russell et al., 2012) but is currently found in a wide variety of estuarine and coastal marine environments across the tropics and subtropics (Canonico et al., 2005).

It is notable not only for its high salinity tolerance (Whittield and Blaber, 1979) but also for its extreme tolerance of hypoxia (Lague et al., 2012), high temperature (Philippart and Ruwet, 1982), and low pH (Krishna Murthy et al., 1984). Despite this eurytolerance, previous studies have revealed sublethal effects including decreased immune functioning, prolonged metabolic impairment, and intracellular pH declines resulting from environmental perturbations that fall well inside the lethal limits (Evans et al., 2003; Van Dijk et al., 1993; Van Ginneken et al., 1999, 1996; Waarde et al., 1990).

We measured the response of this species to environmental perturbation at two levels of organization: blood-borne indicators (blood glucose, base excess, pCO_2, and hemoglobin oxygen saturation) and whole-animal performance (resting and maximum oxygen consumption rates). While blood glucose levels are a commonly used stress indicator (Van Ham et al., 2003), base excess, pCO_2, and hemoglobin oxygen saturation rates track various mechanisms by which changing environmental parameters could impact whole-organism processes. Base excess for example is an indicator of metabolic (i.e. non-respiratory) acid-base disturbance (Siggaard-Andersen and Fogh-Andersen, 1995). Stressors that reduce the capacity for aerobic metabolism, including high temperature or low oxygen, can induce metabolic acidosis by encouraging the production of acid species from anaerobic metabolism (Pörtner, 2002). Blood pCO_2, on the other hand, is an indicator of respiratory acid-base disturbance (Irizarry and Reiss, 2009; Perry and Gilmour, 2002). Increased rates of CO_2 transfer between the blood and environment through the gills (such as during environmental hypercapnia) can alter pCO_2 while leaving base excess relatively unchanged. Both metabolic and respiratory acidoses can alter hemoglobin oxygen affinity through the Bohr effect (Nikinmaa, 2001), while high levels of blood pCO_2

can have more systemic effects including altered neuronal functioning (Chung et al., 2014; Nilsson et al., 2012). Finally, changes in acid-base balance, oxygen utilization, or environmental hypoxia can alter venous hemoglobin oxygen saturation rates, which in turn can affect the capacity for aerobic metabolism (Pörtner et al., 2010).

Many of these perturbations are likely to be reflected in changes to a quantity known as aerobic scope, which is calculated as the difference between maximum and resting aerobic metabolic rates (Fry, 1971). Various investigators have used aerobic scope as an integrative measure of environmental impacts on whole-organism performance, and numerous studies have linked aerobic scope to the ability to perform activities including growth, sustained swimming, feeding, and territorial aggression in a range of active and sedentary species (Claireaux et al., 2000; Claireaux and Lefrançois, 2007; Khan et al., 2014; Killen et al., 2014; Mallekh and Lagardére, 2002; Reidy et al., 2000). While there has been some recent controversy as to the extent to which aerobic scope can be considered a master limiting variable on all physiological performance (Clark et al., 2013), it remains a useful measure of the total metabolic energy available for activities including growth and reproductive processes (Farrell, 2013).

We used aerobic scope as an integrative measure of the impact of simultaneous climate-driven stressors on *O. mossambicus*, and supplemented this metric with the measurement of blood parameters. Our aim was to determine whether temperature, oxygen, and CO_2 impacts interact in an additive, synergistic, or compensatory manner for the magnitudes of perturbation that can be expected to result from end-century climate change.

3.3. Methods

3.3.1. Study Animals

Male seawater-spawned and reared (i.e. freshwater naïve) *Oreochromis mossambicus* (N = 28) of mass 40–80g (mean = 59.9 ± 6.7g) were selected from outdoor tanks maintained at the Hawaii Institute of Marine Biology, University of Hawaii. Although *O. mossambicus* is an estuarine species that is capable of surviving in both seawater and freshwater environments, all experiments were conducted in seawater. Fish were kept under natural photoperiod and ambient seawater temperatures of approximately 24°C.

3.3.2. Custom Climate-Controlled Respirometer Construction

Acrylic Blazka-type swim-tunnel respirometers of 7L internal volume were constructed for this study, following the design employed by Febry and Lutz (1987). Respirometers were submerged in heated water baths to maintain a constant temperature. Intermittent water exchange (i.e. intermittent flow respirometer; Steffensen, 1989) with a UV-sterilized 100L reservoir allowed us to manipulate the temperature, oxygen, and pCO_2 in the respirometer. A Neptune Systems controller combined with custom software automated the release of compressed O_2, N_2, and CO_2 through a gas-exchange column to change gas partial pressures. Two 400W thermostat-controlled submersible heaters were used to set water temperatures in the reservoir and optical oxygen, pH, and temperature probes (Thermo Fisher Scientific, Waltham, MA) were used to monitor the conditions both inside the respirometer and the reservoir. Reservoir pCO_2 was determined using a non-dispersive infrared (NDIR) CO_2 monitor (CO2Meter Inc., Ormond Beach, FL) in the confined headspace at the top of the reservoir.

3.3.3. Selection of Treatment Conditions

Experimental conditions within the respirometer were chosen to represent the upper range of plausible oxygen, temperature, and pCO_2 perturbations that may become prevalent in the oceans due to end-century climate change. Control treatments were chosen based on ambient environmental conditions. The precise target values for each treatment parameter are listed in Table 1. Justifications for these targets are as follows:

Temperature: End-century global sea surface warming projected by Coupled Model Intercomparison Project 5 (CMIP5) models under Representative Concentration Pathway (RCP) 8.5 emissions scenarios (similar to “business as usual” emissions projections) range from 2-4°C (Bopp et al., 2013). We elected for the higher range of temperature change: an increase of 4°C (control temperature 25°C, high temperature stress 29°C). This reflects the fact that although RCP 8.5 was originally intended as a high-emissions scenario, current trends in global emissions match or exceed this trajectory (Sanford et al., 2014).

Oxygen: Shoaling of oxygen minimum layers in the open ocean as well as intensification of coastal hypoxic zones are expected to result from climate-change induced surface-ocean stratification and the intensification of coastal upwelling (Bakun, 1990; Diaz and Rosenberg, 2008; Gilly et al., 2013; Stramma et al., 2011). Though levels of deoxygenation are likely to vary wildly by depth and location, movement of hypoxic waters into previously well-oxygenated habitats could cause substantial declines in oxygen concentration (>50%) in certain sections of both coastal and open waters (Diaz and Rosenberg, 2008; Hofmann and Schellnhuber, 2009; Justin et al., 1996; Oschlies et al., 2008). We chose approximately 50% oxygen saturation (100 µmol O_2/kg) as our low-oxygen treatment. Although this is a fairly drastic decline, we believe it is a useful point of comparison for our study and reflects possible contributions of coastal eutrophication in addition to climate change driven deoxygenation.

CO_2: pCO_2 levels equilibrated to 2x pre-industrial pCO_2 in oxygen minimum zone waters have been predicted to rise in many cases to over 2,000 µatm (Brewer and Peltzer, 2009). Similarly, CMIP5 model results suggest end-century pCO_2 increases in some open-ocean oxygen minimum zones of a similar degree (see Chapter I). Analogously large pCO_2 values in hypoxic coastal zones are also expected to be exacerbated by climate change in conjunction with anthropogenic eutrophication (Cai et al., 2011; Melzner et al., 2012). We chose this range of pCO_2 (2,000 µatm) for our experiments.

3.3.4. Respirometry Protocol

A standard respirometry protocol (Reidy et al., 2000) was used to measure maximum (active) and minimum metabolic rates. Although minimum metabolic rates are often referred to as standard rates, because our study animals were juveniles our minimum metabolic rate measurements likely included some expenditure for growth. Therefore, we use the terminology “minimum metabolism” to distinguish from the strict definition of standard metabolism, which does not include a growth term.

Tilapia were transferred to the respirometer and maintained at rest for twenty-four hours, with a further overnight period of metabolic measurement at rest to determine the minimum metabolism. Current speeds within the respirometer were then slowly increased to 5 body-lengths per second for up to two hours until the fish tired, which was judged to occur when the fish were unable to maintain position in the current and fell to a retaining screen at the back of the swim tunnel. Flow within the swim tunnel was then briefly reversed and restarted to confirm that exhaustion had occurred. The rate of oxygen consumption during the final measurement period prior to exhaustion was taken to be the maximal active metabolic rate, and was calculated according to the following formula:

$$\dot{M}_{O_2} = \left(\frac{\Delta O_{2w}}{\Delta T}\right) \times V \qquad \text{Eq. 1.}$$

where ΔO_{2w} is the change in oxygen concentration during the measurement period, ΔT is the duration of the measurement period, and V is the volume of the respirometer. This protocol was repeated for each fish for all treatments in a randomized sequence. Measured metabolic rates were corrected for body mass scaling effects using a standard scaling exponent of 0.8 (Ros et al., 2006), which is consistent with the seawater mass scaling exponent measured in *O. mossambicus* by Febry and Lutz (1987) and Yamamoto (1992). Bacterial oxygen consumption in the respirometer system measured at the beginning and end of the experimental period by measuring oxygen uptake in the empty respirometer showed negligible bacterial consumption (possibly due to continuous UV-sterilization of the reservoir) and was therefore assumed to be zero.

3.3.5. Blood Sampling

Fish were anesthetized with 0.3 ml/L 2-phenoxyethanol and non-lethal blood draws with heparinized syringes were conducted from the caudal vein. Blood was sampled at the conclusion of the exercise trials and compared against resting controls. Analysis was performed on an i-STAT PCA with the CG4+ cartridge (Abbott Laboratories, Abbott Park, Illinois). All measurements were conducted with blood warmed to 37°C.

3.3.6. Experimental Design

Our study was designed to reveal any interactions between temperature and the additional two environmental parameters. As such, we chose an experimental design that examined oxygen and CO_2 treatments at high (29°C) and low (25°C) temperature. We then compared the effect of warming (low vs. high temperature, a difference of 4°C) under conditions of normoxia and normocapnia, normoxia and hypercapnia, and hypercapnia and hypoxia, respectively. Under this design, a compensatory interaction between temperature, oxygen, and CO_2 would be indicated by a decrease in the magnitude of the warming effect when combined with simultaneous hypoxia or hypercapnia as compared to cases where the warming effect is combined with normoxia and normocapnia. An additive interaction would be indicated by no change in the magnitude of the warming effect, and a synergistic interaction by an increase in the magnitude of the warming effect under O_2 or CO_2 stress. Significance testing of differences between individual treatments was conducted using Tukey's HSD, and the overall trend in the magnitude of the effect of warming with increasing environmental challenge (i.e. control -> hypercapnia -> hypercapnia and hypoxia) was described by performing a linear regression on the data from each fish. The significance of this trend across all fish was tested by determining whether the mean of the slopes of the linear regressions was higher than zero by a single-sample t-test.

3.4. Results

Measured treatment conditions and errors are reported in Table 2. Treatment means were all within ± 2% of the target parameter values.

Measured blood glucose levels ranged from 36 ± 10 mg/dL (resting conditions) to 173 ± 51.3 mg/dL (high-temperature low-oxygen high CO_2 conditions), while base excess (BE) ranged from -22 ± 1 mmol/L (resting) to -29 ± 1 mmol/L (high-temperature) (Figure 1, a and b). Due to limits in the measurement range of our instrument, base excess readings of -30 (9 out of 28 measurements) could not be distinguished from values below -30. Blood pCO_2 ranged from 22800 ± 5400 µatm (resting) to 11000 ± 3100 µatm (high temperature), and venous oxygen saturation (svO_2) ranged from 54 ± 11% (resting) to 17 ± 8% (high-temperature, high CO_2) (Figure 1, c and d).

Comparison of blood parameters between aggregated treatment groups using Tukey's HSD showed that blood glucose was significantly elevated under control and single-stressor treatments relative to resting conditions, and under multiple-stressor treatments relative to control or single-stressor conditions (Figure 2a). Similarly, there was a significant decline in base excess observed between resting and exercise treatments (Figure 2b), as well as a decline in blood pCO_2 under normocapnic exercise compared to resting or hypercapnic treatments (Figure 2c). Finally, venous oxygen saturation was highest at rest, and significantly lower under both hypoxic and normoxic exercise (Figure 2d).

The mean minimum overnight metabolic rate achieved under control conditions was 2.0 ± 0.2 mmol O_2 $kg^{-0.8}$ min^{-1} and was 2.86 ± 0.13 mmol O_2 $kg^{-0.8}$ min^{-1} under elevated temperatures (treatments *Control* and *+T*; Figure 3). Active metabolic rates were 10.28 ± 1.08 O_2 $kg^{-0.8}$ min^{-1} under control conditions and 10.91 ± 1.49 mmol O_2 $kg^{-0.8}$ min^{-1} under elevated temperatures (treatments *Control* and *+T*; Figure 3). Mean aerobic scope was highest under control conditions at 8.27 ± 0.97 mmol O_2 $kg^{-0.8}$ min^{-1} and declined to 3.51 ± 0.77 mmol O_2 $kg^{-0.8}$ min^{-1} under maximally stressful, high-temperature low-oxygen high CO_2 conditions (treatments *Control* and *+CO2/O2/T*; Figure 4).

Comparison of aerobic scope between treatments using Tukey's HSD revealed statistically significant reductions in aerobic scope between the control treatment and all multi-stressor treatments *(+CO2/T, +CO2/O2, +CO2/O2/T*) at $p < 0.05$, but not between the control and single-stressor treatments (*+CO2, +T*; Figure 4). All comparisons of aerobic scope treatments are reported in Table 3.

The mean decrement in aerobic scope resulting from 4°C warming was 0.22 mmol O_2 $kg^{-0.8}$ hr^{-1} in the single-stressor case (comparison of treatments *Control* and *+T*), 0.64 mmol O_2 $kg^{-0.8}$ hr^{-1} in the two-stressor case (comparison of treatments *+CO2* and *+CO2/T*), and 0.93 mmol O_2 $kg^{-0.8}$ hr^{-1} in the three-stressor case (comparison of treatments *+CO2/O2* and *+CO2/O2/T*). The change in the magnitude of the temperature effect for the single, double, and three-stressor case was quantified for each individual fish using linear regressions, and a single-sample t-test of the resulting distribution of slopes was found to be significantly greater than zero ($N = 7$, $p = 0.024$; Figure 5).

3.5. Discussion

3.5.1. Overview

Our results indicate a substantial disparity between individual climate change stressors and multiple simultaneous stressors on the physiological performance of *O. mossambicus*. Aerobic scope values declined significantly in all multiple-stressor trials compared to control conditions but did not decline or showed non-significant declines under both high temperature and high pCO_2 challenge (Figure 4). While high aerobic scope was maintained due to compensatory changes in minimum and active metabolism in the single-stressor trials (i.e. high minimum metabolism in the *+T* trials were compensated by a non-significant rise in active metabolism, low active metabolism in the *+CO2* trials by a decline in minimum metabolism), this compensation appeared to break down under multiple stressors (Figure 3). The ability to resist aerobic scope impairment in response to 4°C temperature warming in particular was significantly degraded as the number of stressors increased—the magnitude of the change is exemplified by an almost 4-fold rise in the effect of temperature in the presence of hypercapnic and hypoxic stress (the difference between *+CO2/O2* and +CO2/O2/T trials) as compared to the single-stressor case (Control versus *+T*) (Figure 5).

Notably, this synergistic interaction between hypercapnic/hypoxic stress and temperature was undetectable when minimum or active metabolic rates were compared instead of aerobic scope. This is important in view of previous work using other metabolic indicators such as routine metabolic rate, which failed to find significant interactions between multiple stressors (pH and hypoxia) on *O. mossambicus* (Van Ginneken et al., 1996). There is no straightforward way to infer aerobic capacity from minimum, routine, or active metabolic rates, and analyses of routine metabolism in particular are complicated by the fact that factors such as behavior or endogenous hormones can have a large impact on the measured metabolism (see Ros et al., 2004 for an example in *O. mossambicus*). Although routine and minimum metabolic rate measurements are a popular metric for evaluating the impact of environmental stressors on marine teleosts, our results emphasize the importance of aerobic scope as potentially a more sensitive metric of environmental stress.

3.5.2. Comparison of Metabolic Rates With Previous Studies

The metabolic rates we measured are broadly consistent with those obtained from previous studies. The minimum metabolic rate we measured under control conditions (2.0 mmol O_2 $kg^{-0.8}$ hr^{-1} at 25°C) is equivalent to the standard metabolic rate reported by Zikos et al. (2014) (2.4 mmol O_2 $kg^{-0.8}$ hr^{-1} at 25°C) and slightly above those measured using *O. mossambicus* X *hornorum* hybrids by Febry and Lutz (1987) (1.8 mmol O_2 $kg^{-0.8}$ hr^{-1} at 28°C). Measurements of maximum metabolic rate for this species, on the other hand, are scarce, and a "maximum routine metabolic rate" reported by Ros et al. (2004) (4.4 mmol O_2 $kg^{-0.8}$ hr^{-1} at 26°C) should be considered to be an underestimate of maximum metabolism because it was measured during spontaneous activity rather than during a swimming or exhaustion trial. Similarly, while the "recovery metabolism" reported by Schnell and Seebacher (2008) was taken post-exhaustion, the fact that their reported recovery metabolic rates (1.9 mmol O_2 $kg^{-0.8}$ hr^{-1} at 26°C) often fall below what they report to be minimum metabolic rates strongly suggests that their measurements missed the peak excess post-exercise oxygen consumption (EPOC) and are therefore substantial underestimates of peak recovery metabolism. A swim-tunnel respirometry trial of *O. mossambicus* X *hornorum* hybrids by McKenzie et al. (2003) did generate substantially higher maximum metabolic rates (19.2 mmol O_2 $kg^{-0.8}$ hr^{-1} at 25°C) than in our study, however it is difficult to determine whether this higher rate was due to differences in the fish stock (hybrid tilapia versus *O. mossambicus*), water parameters (McKenzie et al. conducted their experiments in freshwater), or other factors. The unusually high minimum metabolic rates reported in the McKenzie et al. study (3.2 mmol O_2 $kg^{-0.8}$ hr^{-1} at 25°C) do suggest however that their fish may have had a more active aerobic metabolism than those used in ours. Moreover, an earlier study (also in freshwater) on the same *mossambicus* X *hornorum* hybrids by Febry and Lutz (1987) measured active metabolic rates between 8–13 mmol O_2 $kg^{-0.8}$ hr at 28°C, which closely matches our results.

Recent studies have shown that some teleosts may achieve higher metabolic rates when exhausted under postprandial conditions than in a fasted state (Clark et al., 2013; Fu et al., 2009), although these metabolic rates may converge under aerobic stress (Jourdan-Pineau et al., 2010). Unfortunately, no measurements of postprandial maximum metabolic rate have been reported in *O. mossambicus* to date, and therefore it is untenable to compare our measurements with those obtained from fed fish. Measurements do exist in similarly sized (40–80 g) postprandial males of the congeneric Nile tilapia (*Oreochromis niloticus*) however (Mamun et al., 2015), and these suggest that postprandial maximum metabolic rates (9 mmol O_2 $kg^{-0.8}$ min^{-1}) are comparable to those obtained in our study.

3.5.3. Blood-Borne Stress Indicators

Our blood analysis bolsters our metabolic rate analysis by revealing additional evidence of physiological stress under increasing environmental challenge. Elevated blood glucose levels suggest significantly higher stress under multiple-stressor as opposed to single-stressor and control treatments (Figure 2). The magnitude of the change in blood glucose between resting and maximal stress conditions is consistent with previous research on the effect of exercise and environmental stressors on teleost glucose levels including interactions between temperature and exercise in turbot (*Scophthalmus maximus*; Van Ham et al., 2003), temperature and salinity in *O. mossambicus* (Fiess et al., 2007; Zaragoza et al., 2008) and dissolved oxygen (Evans et al., 2003). Significantly elevated blood pCO_2 under hypercapnic as opposed to normocapnic exercise indicates a respiratory acidosis caused by increased CO_2 diffusion from the environment, while significantly lower pCO_2 under normocapnic exercise as opposed to resting conditions can be attributed to respiratory alkalosis caused by increased ventilatory frequency. The high blood pCO_2 observed under hypercapnia is also likely to have been driven in part by the metabolic acidosis caused by exhaustive exercise, which is indicated by more negative base excess under all exercise treatments. It is important to point out that the common measurement temperature of 37°C (regulated by the i-STAT analyzer) means that our dataset is not likely to be reflective of *in vivo* conditions. Previous measurements of pCO_2 in freshwater-reared *O. mossambicus* for example show partial pressures of approximately 4,000 µatm (although the text does not specify whether measurements were performed in the dorsal or ventral aorta; Smit et al., 1981). However, studies across a variety of teleosts have indicated that relative differences in measured values are meaningful even under this elevated temperature (see Stoot et al., 2014, for a review).

3.5.4. Implications for Climate Change Impacts

Our experimental treatments were limited to acute (<36 hours) stressors, whereas climate change stressors are chronic, multi-generational perturbations that allow affected species to mitigate the impacts on physiological functioning through gene regulatory responses, epigenetic inheritance, or genetic evolution (Somero, 2011). Studies on *O. mossambicus* have shown substantial ability to acclimate to large (8°C) temperature shifts over a period of several weeks (Schnell and Seebacher, 2008). Conversely, declining *O. mossambicus* populations in the Salton Sea in response to incremental salinity increases (approx. 0.25ppt/year) over a forty-year period attest to the limits of these adaptive processes (Caskey et al., 2007; Hurlbert et al., 2007). Similarly, Ginneken et al. (1996) reported that *O. mossambicus* exhibited no adaptation response to 6-weeks of environmental acidosis. Unfortunately, logistical limitations prevented us from evaluating the ability of our animals to acclimate to the treatment conditions we imposed in this study.

Nevertheless, it is possible to draw some general conclusions from our results for predicting teleost responses to climate change. The significant impact of simultaneous stressors on our study animals was particularly surprising in the context of the resilience of this species to single-stressor perturbations, both in our experiments and in the field. The thermal tolerance zone of *O. mossambicus* in the hypersaline environment of the Salton Sea is reported to range from 10–35°C (Costa-Pierce and Doyle, 1997) for example, with some studies reporting lethal limits as low as 8°C (Stauffer, 1986) and as high as 42°C (Philippart and Ruwet, 1982). Hypercapnia and hypoxia tolerance is likewise very high, with acidic lethal limits (measured in acid-titration experiments, not CO_2-addition) in freshwater as low as pH 3.4 (Krishna Murthy et al., 1984), and a survivable oxygen limit down to 20% saturation (Rappaport et al., 1976). The existence of measurable sublethal effects in our experiments on such a putatively "hardy" organism portends similar synergistic impacts in other marine teleosts. CMIP5 simulations of end-century climate change underscore the correlation between these climate-related parameters, with projections under an RCP 8.5 emissions scenario showing a 0.127 unit decline in global mean surface pH and a 1.3% decline in global oxygen content for every °C increase in sea surface temperature (Bopp et al., 2013).

3.5.5. Conclusions and Directions for Further Study

The existing literature has made large strides in understanding the impact of one or two stressors in combination (Claireaux et al., 2000; Ginneken et al., 1996; Ishimatsu et al., 2008; Lefrançois and Claireaux, 2003; Munday et al., 2009) as well as on integrating species-level changes to an ecosystem context (Lehodey et al., 2008; Link et al., 2010). While these are important endeavors, our results show that the impact of temperature, oxygen, and CO_2 together may exceed even that predicted by these studies. More experiments on a finer range of environmental conditions and a variety of teleost species will further contribute to our understanding. In particular, we hope that our results may serve as a baseline against which the effect of chronic stressors (i.e. long-term acclimation or the rearing of multiple generations under environmental challenge) could be compared to evaluate the capacity for adaptation driven by short-term physiological plasticity and epigenetic change.

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3.7. References

Adams, S.M., 2005. Assessing cause and effect of multiple stressors on marine systems. Mar. Pollut. Bull. 51, 649–657. doi:10.1016/j.marpolbul.2004.11.040

Bakun, A., 1990. Global climate change and intensification of coastal ocean upwelling. Science (80-.). 247, 198–201. doi:10.1126/science.247.4939.198

Bopp, L., Resplandy, L., Orr, J.C., Doney, S.C., Dunne, J.P., Gehlen, M., Halloran, P., Heinze, C., Ilyina, T., Séférian, R., Tjiputra, J., Vichi, M., 2013. Multiple stressors of ocean ecosystems in the 21st century: projections with CMIP5 models. Biogeosciences 10, 6225–6245. doi:10.5194/bg-10-6225-2013

Brewer, P.G., Peltzer, E.T., 2009. Limits to Marine Life. Science (80-.). 324, 347–348.

Cai, W.-J., Hu, X., Huang, W.-J., Murrell, M.C., Lehrter, J.C., Lohrenz, S.E., Chou, W.-C., Zhai, W., Hollibaugh, J.T., Wang, Y., Zhao, P., Guo, X., Gundersen, K., Dai, M., Gong, G.-C., 2011. Acidification of subsurface coastal waters enhanced by eutrophication. Nat. Geosci. 4, 766–770. doi:10.1038/ngeo1297

Canonico, G.C., Arthington, A., Mccrary, J.K., Thieme, M.L., 2005. The efects of introduced tilapias on native biodiversity. Aquat. Conserv. Mar. Freshw. Ecosyst. 15, 463–483. doi:10.1002/aqc.699

Caskey, L.L., Riedel, R.R., Costa-Pierce, B., Butler, J., Hurlbert, S.H., 2007. Population dynamics, distribution, and growth rate of tilapia (Oreochromis mossambicus) in the Salton Sea, California, with notes on bairdiella (Bairdiella icistia) and orangemouth corvina (Cynoscion xanthulus). Hydrobiologia 576, 185–203. doi:10.1007/s10750-006-0301-2

Chung, W.-S., Marshall, N.J., Watson, S.-A., Munday, P.L., Nilsson, G.E., 2014. Ocean acidification slows retinal function in a damselfish through interference with GABAA receptors. J. Exp. Biol. 217, 323–6. doi:10.1242/jeb.092478

Claireaux, G., Lefrançois, C., 2007. Linking environmental variability and fish performance: integration through the concept of scope for activity. Philos. Trans. R. Soc. B 362, 2031–2041. doi:10.1098/rstb.2007.2099

Claireaux, G., Webber, D.M., Lagardére, J.P., Kerr, S.R., 2000. Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (Gadus morhua). J. Sea Res. 44, 257–265. doi:10.1016/S1385-1101(00)00053-8

Clark, T.D., Sandblom, E., Jutfelt, F., 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. J. Exp. Biol. 216, 2771–2782. doi:10.1242/jeb.084251

Cooke, S.J., Hinch, S.G., Farrell, a. P., Patterson, D. a., Miller-Saunders, K., Welch, D.W., Donaldson, M.R., Hanson, K.C., Crossin, G.T., Mathes, M.T., Lotto, a. G., Hruska, K. a., Olsson, I.C., Wagner, G.N., Thomson, R., Hourston, R., English, K.K., Larsson, S., Shrimpton, J.M., Van der Kraak, G., 2008. Developing a Mechanistic Understanding of Fish Migrations by Linking Telemetry with Physiology, Behavior, Genomics and Experimental Biology: An Interdisciplinary Case Study on Adult Fraser River Sockeye Salmon. Fisheries 33, 321–339. doi:10.1577/1548-8446-33.7.321

Costa-Pierce, B.A., Doyle, R.W., 1997. Genetic identification and status of tilapia regional strains in southern California. Tilapia Aquac. Am. 1, 1–17.

Crain, C.M., Kroeker, K., Halpern, B.S., 2008. Interactive and cumulative effects of multiple human stressors in marine systems. Ecol. Lett. 11, 1304–15. doi:10.1111/j.1461-0248.2008.01253.x

Cruz-Neto, A.P., Steffensen, J.F., 1997. The effects of acute hypoxia and hypercapnia on oxygen consumption of the freshwater European eel. J. Fish Biol. 50, 759–769. doi:10.1006/jfbi.1996.0337

Diaz, R.J., Rosenberg, R., 2008. Spreading dead zones and consequences for marine ecosystems. Science (80-.). 321, 926–929. doi:10.1126/science.1156401

Evans, J.J., Shoemaker, C. a, Klesius, P.H., 2003. Effects of sublethal dissolved oxygen stress on blood glucose and susceptibility to Streptococcus agalactiae in Nile tilapia Oreochromis niloticus. J. Aquat. Anim. Health 15, 202–208.

doi:10.1577/H03-024

Farrell, A.P., 2013. Aerobic scope and its optimum temperature: clarifying their usefulness and limitations – correspondence on J. Exp. Biol. 216, 2771-2782. J. Exp. Biol. 216, 4493 LP-4494.

Farrell, A.P., Hinch, S.G., Cooke, S.J., Patterson, D.A., Crossin, G.T., Lapointe, M., Mathes, M.T., 2008. Pacific Salmon in Hot Water: Applying Aerobic Scope Models and Biotelemetry to Predict the Success of Spawning Migrations. Physiol. Biochem. Zool. 81. doi:10.1086/592057

Febry, B.Y.R., Lutz, P., 1987. Energy Partitioning in Fish: the Activity- Related Cost of Osmoregulation in a Euryhaline Cichlid. J. Exp. Biol. 128, 63–85.

Fiess, J.C., Kunkel-patterson, A., Mathias, L., Riley, L.G., Yancey, P.H., Hirano, T., Grau, E.G., 2007. Effects of environmental salinity and temperature on osmoregulatory ability, organic osmolytes, and plasma hormone profiles in the Mozambique tilapia (Oreochromis mossambicus). Comp. Biochem. Physiol. Part A 146, 252–264. doi:10.1016/j.cbpa.2006.10.027

Fry, F.E.J., 1971. The Effect of Environmental Factors on the Physiology of Fish, in: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology. Academic Press, New York, pp. 1–98.

Fu, S., Zeng, L.-Q., Li, X.-M., Pang, X., Cao, Z.-D., Peng, J.-L., Wang, Y.-X., 2009. Effect of meal size on excess post-exercise oxygen consumption in fishes with different locomotive and digestive performance. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 179, 509–517. doi:10.1007/s00360-008-0337-x

Gilly, W.F., Beman, J.M., Litvin, S.Y., Robison, B.H., 2013. Oceanographic and Biological Effects of Shoaling of the Oxygen Minimum Zone. Ann. Rev. Mar.

Sci. 5, 393–420. doi:10.1146/annurev-marine-120710-100849

Ginneken, V. Van, Thillart, G. Van Den, Addink, A., Erkelens, C., 1996. Synergistic effect of acidification and hypoxia: in vivo 31P-NMR and respirometric study in fishes. Am. J. Physiol. 271, 1746–1752.

Hofmann, M., Schellnhuber, H.-J., 2009. Oceanic acidification affects marine carbon pump and triggers extended marine oxygen holes. Proc. Natl. Acad. Sci. U. S. A. 106, 3017–22. doi:10.1073/pnas.0813384106

Hurlbert, A.H., Anderson, T.W., Sturm, K.K., Hurlbert, S.H., 2007. Fish and fish-eating birds at the Salton Sea: a century of boom and bust. Lake Reserv. Manag. 23, 469–499.

Irizarry, R., Reiss, A., 2009. Arterial and Venous Blood Gases: Indications, Interpretations, and Clinical Applications. Compendium October, 1–7.

Ishimatsu, A., Hayashi, M., Kikkawa, T., 2008. Fishes in high-CO2, acidified oceans. Eff. Ocean Acidif. Mar. Ecosyst. 373, 295–302.

Jourdan-Pineau, H., Dupont-Prinet, A., Claireaux, G., Mckenzie, D.J., 2010. An Investigation of Metabolic Prioritization in the European Sea Bass , Dicentrarchus labrax An Investigation of Metabolic Prioritization in the European. Physiol. Biochem. Zool. 83. doi:10.1086/648485

Justin, D., Rabalais, N.N., Turner, R.E., 1996. Effects of climate change on hypoxia in coastal waters: A doubled CO2 scenario for the northern Gulf of Mexico. Limnol. Oceanogr. 41, 992–1003. doi:10.4319/lo.1996.41.5.0992

Khan, J.R., Pether, S., Bruce, M., Walker, S.P., Herbert, N.A., 2014. Optimum temperatures for growth and feed conversion in cultured hapuku (Polyprion oxygeneios) - Is there a link to aerobic metabolic scope and final temperature preference? Aquaculture 430, 107–113. doi:10.1016/j.aquaculture.2014.03.046

Killen, S.S., Mitchell, M.D., Rummer, J.L., Chivers, D.P., Ferrari, M.C.O., Meekan, M.G., McCormick, M.I., 2014. Aerobic scope predicts dominance during early life in a tropical damselfish. Funct. Ecol. In press. doi:10.1111/1365-2435.12296

Krishna Murthy, V., Dhananjaya Reddy, Y., Manohar Reddy, R., Bhaskar, M., Govindappa, S., 1984. Changes in brain acetylcholinesterase activity and behaviour of freshwater fish in acid polluted environment. Environ. Ecol. 2, 79–82.

Lague, S.L., Speers-Roesch, B., Richards, J.G., Farrell, A.P., 2012. Exceptional cardiac anoxia tolerance in tilapia (Oreochromis hybrid). J. Exp. Biol. 215, 1354–65. doi:10.1242/jeb.063362

Lapointe, D., Vogelbein, W.K., Fabrizio, M.C., Gauthier, D.T., Brill, R.W., 2014. Temperature, hypoxia, and mycobacteriosis: effects on adult striped bass Morone saxatilis metabolic performance. Dis. Aquat. Organ. 108, 113–27. doi:10.3354/dao02693

Lefrançois, C., Claireaux, G., 2003. Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole Solea solea. Mar. Ecol. Prog. Ser. 259, 273–284.

Lehodey, P., Senina, I., Murtugudde, R., 2008. A spatial ecosystem and populations dynamics model (SEAPODYM) – Modeling of tuna and tuna-like populations. Prog. Oceanogr. 78, 304–318. doi:10.1016/j.pocean.2008.06.004

Link, J.S., Fulton, E.A., Gamble, R.J., 2010. The northeast US application of ATLANTIS: A full system model exploring marine ecosystem dynamics in a living marine resource management context. Prog. Oceanogr. 87, 214–234. doi:10.1016/j.pocean.2010.09.020

Mallekh, R., Lagardére, J.P., 2002. Effect of temperature and dissolved oxygen concentration on the metabolic rate of the turbot and the relationship between metabolic scope and feeding demand. J. Fish Biol. 60, 1105–1115. doi:10.1006/jfbi.2002.1918

Mamun, S.M., Focken, U., Becker, K., 2015. Comparison of metabolic rates and feed nutrient digestibility in conventional , genetically improved (GIFT) and ... doi:10.1016/j.cbpa.2007.04.007

McKenzie, D.J., Martinez, R., Morales, A., Acosta, J., Morales, R., Taylor, E.W., Steffensen, J.F., Estrada, M.P., 2003. Effects of growth hormone transgenesis on metabolic rate, exercise performance and hypoxia tolerance in tilapia hybrids. J. Fish Biol. 63, 398–409. doi:10.1046/j.1095-8649.2003.00162.x

Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. a., Bange, H.W., Hansen, H.P., Körtzinger, A., 2012. Future ocean acidification will be amplified by hypoxia in coastal habitats. Mar. Biol. 160, 1875–1888. doi:10.1007/s00227-012-1954-1

Munday, P.L., Crawley, N.E., Nilsson, G.E., 2009. Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. Mar. Ecol. Prog. Ser. 388, 235–242. doi:10.3354/meps08137

Nikinmaa, M., 2001. Haemoglobin function in vertebrates: evolutionary changes in cellular regulation in hypoxia. Respir. Physiol. 128, 317–29.

Nilsson, G.E., Crawley, N., Lunde, I.G., Munday, P.L., 2009. Elevated temperature reduces the respiratory scope of coral reef fishes. Glob. Chang. Biol. 15, 1405–

1412. doi:10.1111/j.1365-2486.2008.01767.x

Nilsson, G.E., Dixson, D.L., Domenici, P., McCormick, M.I., Sørensen, C., Watson, S., Munday, P.L., 2012. Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. Nat. Clim. Chang. 1–4. doi:10.1038/NCLIMATE1352

Oschlies, A., Schulz, K.G., Riebesell, U., Schmittner, A., 2008. Simulated 21st century's increase in oceanic suboxia by CO2-enhanced biotic carbon export. Global Biogeochem. Cycles 22, n/a-n/a. doi:10.1029/2007GB003147

Perry, S.F., Gilmour, K.M., 2002. Sensing and Transfer of Respiratory Gases at the Fish Gill. J. Exp. Zool. 263, 249–263. doi:10.1002/jez.10129

Philippart, J., Ruwet, J.-C., 1982. Ecology and distribution of tilapias, in: Pullin, R.S. V, Lowre-McConnel, R.H. (Eds.), Biology and Culture of Tilapias. ICLARM, Manila, Philippines, pp. 15–59.

Pörtner, H.-O., 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88, 137–146. doi:10.1007/s001140100216

Pörtner, H.-O., 2002. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 132, 739–61.

Pörtner, H.-O., 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. J. Exp. Biol. 213, 881–93. doi:10.1242/jeb.037523

Pörtner, H., Bickmeyer, U., Bleich, M., Bock, C., Brownlee, C., Melzner, F., Michaelidis, B., Sartoris, F., Storch, D., 2010. 9: Studies of acid-base status and regulation, in: Riebesell, U., Fabry, V.J., Hansson, L., Gattuso, J.-P. (Eds.), Guide to Best Practices for Ocean Acidification Research and Data Reporting. Publications Office of the European Union, Luxembourg, pp. 137–166.

Rappaport, A., Sarig, S., Marek, M., 1976. Results of tests of various aeration systems on the oxygen regime of Genosar experimental ponds and growth of fish there in 1975. Bamidgeh 28, 35–49.

Reidy, S.P., Kerr, S.R., Nelson, J.A., 2000. Aerobic and anaerobic swimming performance of individual Atlantic cod. J. Exp. Biol. 203, 347–57.

Ros, A.F.H., Becker, K., Canario, A.V.M., Oliveira, R.F., 2004. Androgen levels and energy metabolism in Oreochromis mossambicus. J. Fish Biol. 65, 895–905. doi:10.1111/j.1095-8649.2004.00484.x

Ros, A.F.H., Becker, K., Oliveira, R.F., 2006. Aggressive behaviour and energy metabolism in a cichlid fish, Oreochromis mossambicus. Physiol. Behav. 89, 164–70. doi:10.1016/j.physbeh.2006.05.043

Rosa, R., Seibel, B. a, 2008. Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. Proc. Natl. Acad. Sci. U. S. A. 105, 20776–80. doi:10.1073/pnas.0806886105

Russell, D.J., Thuesen, P.A., Thomson, F.E., 2012. A review of the biology, ecology, distribution and control of Mozambique tilapia, Oreochromis mossambicus (Peters 1852) (Pisces: Cichlidae) with particular emphasis on invasive Australian populations. Rev. Fish Biol. Fish. 22, 533–554. doi:10.1007/s11160-011-9249-z

Sanford, T., Frumhoff, P.C., Luers, A., Gulledge, J., 2014. The climate policy narrative for a dangerously warming world. Nat. Clim. Chang. 4, 164–166.

doi:10.1038/nclimate2148

Schnell, A.K., Seebacher, F., 2008. Can phenotypic plasticity facilitate the geographic expansion of the tilapia Oreochromis mossambicus? Physiol. Biochem. Zool. 81, 733–742. doi:10.1086/592027

Siggaard-Andersen, O., Fogh-Andersen, N., 1995. Base excess or buffer base (strong ion difference) as measure of a non-respiratory acid-base disturbance. Acta Anaesthesiol Scand 39, 123–128.

Smit, G.L., Hattingh, J., Ferreira, J.T., 1981. The physiological responses of blood during thermal adaptation in three freshwater species. J. Fish Biol. 19, 147–160.

Somero, G.N., 2011. The Physiology of Global Change: Linking Patterns to Mechanisms. Ann. Rev. Mar. Sci. 4, 110301100421037. doi:10.1146/annurev-marine-120710-100935

Stauffer, J.R.J., 1986. Effects of salinity on preferred and lethal temperatures of Mozambique tilapia, Oreochromis Mossambicus (Peters). Water Resour. Bull. 22, 205–208.

Steffensen, J.F., 1989. Some errors in respirometery of aquatic breathers: how to avoid and correct for them. Fish Physiol. Biochem. 6, 49–59.

Stoot, L.J., Cairns, N.A., Cull, F., Taylor, J.J., Jeffrey, J.D., Morin, F., Mandelman, J.W., Clark, T.D., Cooke, S.J., 2014. Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: a review. Conserv. Physiol. 2, cou011. doi:10.1093/conphys/cou011.

Stramma, L., Prince, E.D., Schmidtko, S., Luo, J., Hoolihan, J.P., Visbeck, M., Wallace, D.W.R., Brandt, P., Körtzinger, A., 2011. Expansion of oxygen minimum zones may reduce available habitat for tropical pelagic fishes. Nat. Clim. Chang. 2, 33–37. doi:10.1038/nclimate1304

Van Dijk, P.L.M., Van den Thillart, G.E.E.J.M., Wendelaar Bonga, S.E., 1993. The Influence of Gradual Water Acidification on the Oxigen Consumption Pattern of Fish. Comp. Biochem. Physiol. 105C, 421–427.

Van Ginneken, V.J.T., Addink, A.D.F., Van Den Thillart, G.E.E.J.M., 1996. Direct calorimetry of aquatic animals: Effects of the combination of acidification and hypoxia on the metabolic rate of fish. Thermochim. Acta 276, 7–15. doi:10.1016/0040-6031(95)02778-5

Van Ginneken, V.J.T., Van Den Thillart, G.E.E.J.M., Muller, H.J., Van Deursen, S., Onderwater, M., Visee, J., Hopmans, V., Van Vliet, G., Nicolay, K., 1999. Phosphorylation state of red and white muscle in tilapia during graded hypoxia: an in vivo 31 P-NMR study. Am. J. Physiol. 277, R1501–R1512.

Van Ham, E.H., Van Anholt, R.D., Kruitwagen, G., Imsland, A.K., Foss, A., Sveinsbø, B.O., FitzGerald, R., Parpoura, A.C., Stefansson, S.O., Wendelaar Bonga, S.E., 2003. Environment affects stress in exercised turbot. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 136, 525–538. doi:10.1016/S1095-6433(03)00083-7

Waarde, B.Y.A.V.A.N., Dijk, P.V.A.N., Den, G.V.A.N., Bonga, W., Addink, A., 1990. P-NMR Studies on Acid-Base Balance and Energy Metabolism of Acid-Exposed Fish. J. Exp. Biol. 154, 223–236.

Whittield, A.K., Blaber, S.J.M., 1979. The distribution of the freshwater cichlid Sarotherodon mossambicus in estuarine systems Tilapia , Fish , Estuaries , Habitat , Salinity , Wa- ter currents , Competition , Predation 4, 77–81.

Yamamoto, K.-I., 1992. Relationship of respiration to body weight in the tilapia Oreochromis niloticus under resting and normoxic conditions. Comp. Biochem. Physiol. - A Physiol. 103, 81–83.

Zaragoza, O.D.R., Rodríguez, M.H., Bückle Ramirez, L.F., 2008. Thermal stress effect on tilapia *Oreochromis mossambicus* (Pisces: Cichlidae) blood parameters. Mar. Freshw. Behav. Physiol. 41, 79–89. doi:10.1080/10236240801896223

Zikos, A., Seale, A.P., Lerner, D.T., Grau, E.G., Korsmeyer, K.E., 2014. Effects of salinity on metabolic rate and branchial expression of genes involved in ion transport and metabolism in Mozambique tilapia (Oreochromis mossambicus). Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 178, 121–131. doi:10.1016/j.cbpa.2014.08.016

3.8. Figures and Tables

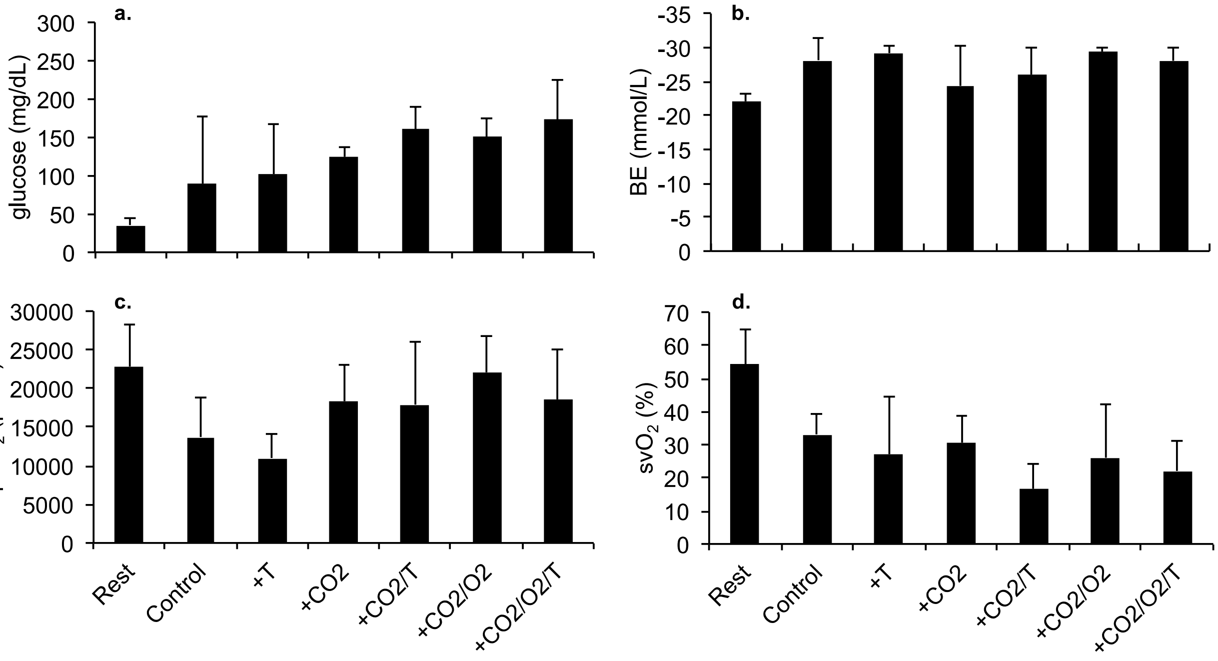

Figure 1: Comparison of blood parameters across experimental treatments. All measurements performed with i-STAT clinical analyzer on venous blood at temperature 37°C. BE = base excess. svO2 = venous oxygen saturation. Error bars show 1 standard deviation. N = 4 for each treatment.

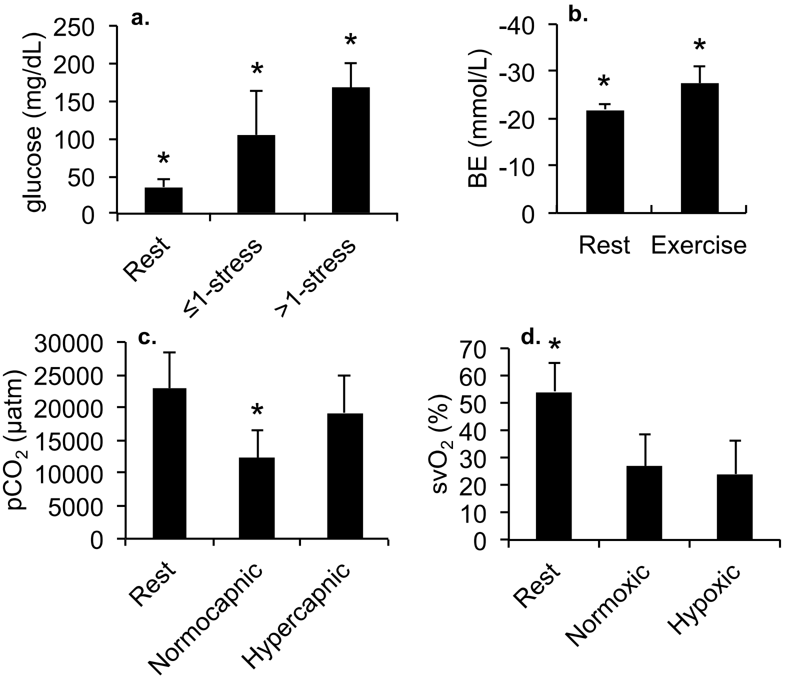

Figure 2: Comparison of blood parameters grouped for statistical comparison. All measurements performed with i-STAT clinical analyzer on venous blood at temperature 37°C. BE = base excess. svO_2 = venous oxygen saturation. Error bars show 1 standard deviation. Categories marked by asterisks are significantly different at $p < 0.05$.

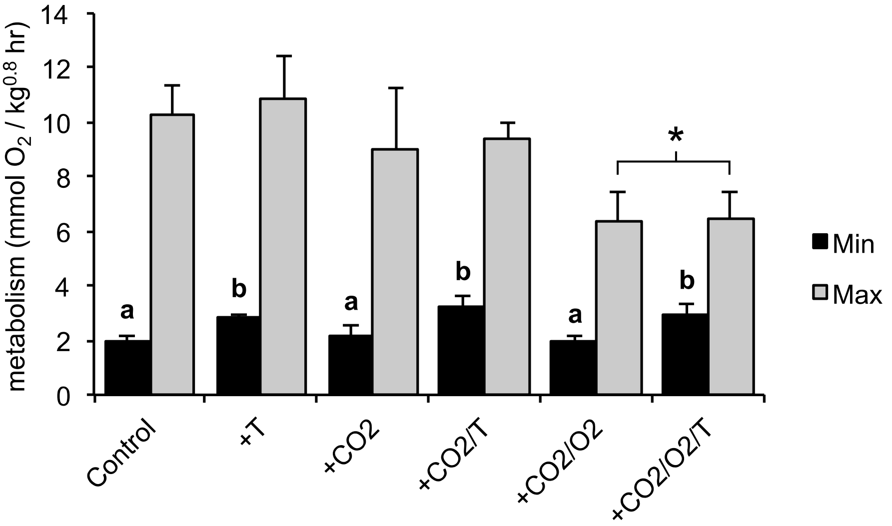

Figure 3: Impact of temperature, oxygen and CO_2 perturbations on active (grey) and minimum (black) metabolic rates of *O. mossambicus*. Means with different letters (a-

b, *) are significantly different (Tukey's HSD, $p < 0.05$). Error bars are ±1 standard deviation. N = 7 for each treatment. Treatment labels correspond to those in Table 1.

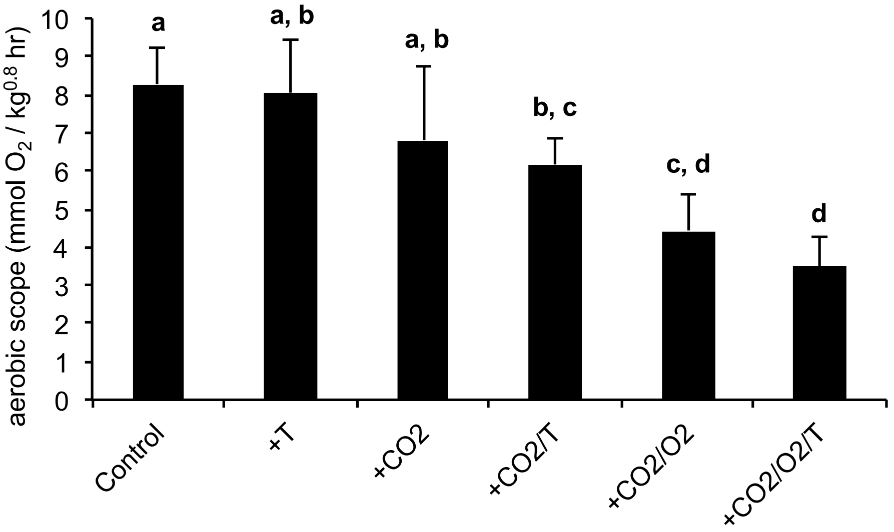

Figure 4: Impact of temperature, oxygen, and CO_2 perturbations on aerobic scope of *O. mossambicus*. Means with different letters (a-d) are significantly different (Tukey's HSD, $p < 0.05$; see Table 3). Error bars are ±1 standard deviation. N = 7 for each treatment. Treatment labels correspond to those in Table 1.

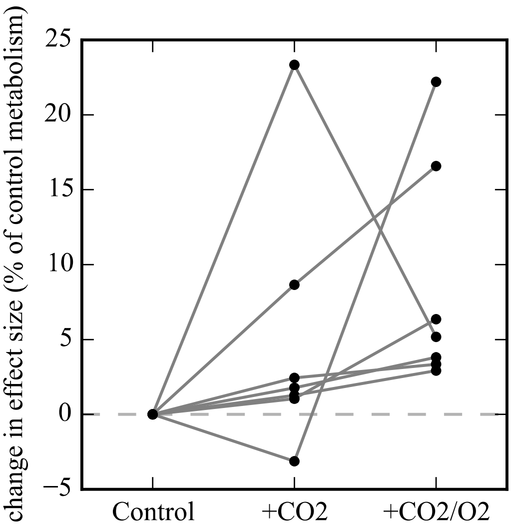

Figure 5: Effect of simultaneous stressors on in the effect of a 4°C warming on aerobic scope. Negative numbers indicate a smaller magnitude effect while positive

numbers indicate increased magnitude. X-axis labels correspond to the treatment labels as in Table 1, and indicate a comparison between high and normal temperature under normocapnia and normoxia (Control), in the presence of hypercapnia (+CO2) and under hypercapnia and hypoxia (+CO2/O2), respectively. Lines indicate individual fishes. In 6 out of 7 cases, the change in aerobic scope due to 4°C warming was greater in the presence of simultaneous CO_2 stress (*+CO2*) than in the control case. In all cases, the change in aerobic scope due to warming was greater in the presence of simultaneous CO_2 and oxygen stress (*+CO2/O2*) than in the control case. The distribution of the slopes of linear regressions performed on each fish are significantly greater than zero by single-sample t-test (p = 0.024, N = 7).

Table 1: Target environmental conditions for experimental treatments.

Treatment	Temperature (°C)	DO (μmol/kg)	pCO_2(μatm)
Control	25.0	200	390
+T	29.0	200	390
+CO2	25.0	200	2000
+CO2/T	29.0	200	2000
+CO2/O2	25.0	100	2000
+CO2/O2/T	29.0	100	2000

Table 2: Measured environmental conditions for experimental treatments. Errors indicate one standard deviation.

Parameter	Routine	Stressful
Temperature (°C)	25.1 ± 0.1	29.1 ± 0.1

DO (µmol/kg)	201.3 ± 6.7	102.0 ± 9.5
pCO$_2$(µatm)	381.4 ± 46.0	2034 ± 54.6

Table 3: Significance testing of pairwise comparisons using Tukey’s HSD. Treatment labels correspond to column labels in Table 1. * Indicates $p < 0.05$. ** Indicates $p < 0.01$.

	+T	+CO2	+CO2/T	+CO2/O2	+CO2/O2/T
Control	0.90	0.30	*0.04	**0.00	**0.00
+T		0.48	0.09	**0.00	**0.00
+CO2			0.90	*0.02	**0.00
+CO2/T				0.15	*0.01
+CO2/O2					0.73

4. An Empirically Derived Index for Predicting the Impacts of Multi-Dimensional Environmental Stress on Marine Teleosts

4.1.1. Abstract

Habitat suitability indices play a key role in many fields of conservation biology from fisheries models to ecosystem modeling. Yet commonly used indices often suffer from weaknesses in predicting habitat suitability in novel or uncommon environments, as well as in disentangling the effects of highly correlated environmental variables. We derive a new index from measurements of a key physiological indicator (aerobic scope) and demonstrate its use in a model teleost, *Oreochromis mossambicus*. We show how this new index can be used to evaluate previously developed theory-based models and as well as to project the possible impacts of anthropogenic environmental change on an area of *O. mossambicus* habitat. Our results are promising for predicting the responses of a wide range of teleosts to future environmental perturbations such as climate change and coastal eutrophication.

4.1.2. Introduction

4.1.3. Habitat Suitability Index: A Tool for Managers and Forecasters

The relationship between a species' distribution and its environment is central to the study of ecology (Guisan and Zimmermann, 2000). It is no surprise then, that models that attempt to predict the spatial distribution of populations in relation to environmental factors are fundamental to many fields (Hirzel et al., 2006; Peterson, 2006). Many of these models take the approach of a "habitat suitability index": an index that takes environmental conditions as inputs and outputs a probability of habitation under those conditions (Hirzel and Le Lay, 2008). Habitat suitability indices are used, among other purposes, for managing exploited species, assessing biotic impacts of pollutants and climate change, analyzing population viability, assessing invasion risk, and predicting human-wildlife conflicts (Brooks, 1997; Hirzel et al., 2001, 2006; Peterson, 2006).

In the marine environment, variants of the habitat suitability index are widespread in fisheries models that attempt to estimate stock abundance from catch and effort data. Maunder, Hinton and Bigelow (Maunder et al., 2006) for example, use a “habitat effect” variable in their fisheries model that describes the effect of environmental conditions on the catchability of a species at a given hook:

$$C = q_{base} H(t_{water}) E N_t \qquad \text{Eq. 1.}$$

In this equation, H is in effect a habitat suitability index that uses physical parameters (water temperature in this example) to predict the likelihood of habitation in a particular water mass, thereby correcting q_{base} (the default catchability across all habitats) to predict C (the catch at a given hook) by taking into account fishing effort E and a time-dependent overall species abundance term N_t.

Similarly, end-to-end ecosystem models, which are rapidly gaining traction as a primary method of predicting climate and fisheries impacts on higher trophic level species (Travers et al., 2007), often rely on habitat suitability indices to relate projected environmental conditions to local population abundance. Lehodey, Senina and Murtugudde (Lehodey et al., 2008) for example, use a “feeding habitat index” in their SEAPODYM (Spatial Ecosystem and Populations Dynamics) model for pelagic tunas that indicates the most favorable areas for habitation outside of the spawning season:

$$H = \Theta(t_{water}, O_{2water})\rho \tag{Eq. 2.}$$

where H is the feeding habitat index, Θ is the accessibility index, and ρ is the prey density. In this case, Θ is a habitat suitability index that describes the likelihood of habitation of a species as a function of water temperature and dissolved oxygen.

This ubiquity of habitat suitability indices means that endeavors ranging from fisheries assessment to ecosystem modeling depend on certain basic assumptions, and in some cases, share certain weaknesses. Our objective is to develop a habitat suitability index that addresses some of these weaknesses: specifically, those that arise with regards to predicting species distributions under scenarios of future environmental change.

4.1.4. Constructing an Index for Future Habitats

Habitat suitability modeling in the context of environmental change presents a unique set of challenges. Two of the most fundamental are (1) correlations between explanatory variables (Prince and Goodyear, 2006; Schmittner et al., 2008) and (2) model extrapolation to novel habitats (Kearney and Porter, 2009; Kearney et al., 2008). Current habitat suitability indices, which largely consist of statistical correlations between presence/absence data and observed environmental parameters, are ill equipped to deal with these challenges (Hirzel and Le Lay, 2008; Homyack, 2010; Kearney and Porter, 2009; Thuiller, 2004).

The problem of the correlation in explanatory variables is strongly related to the fact that common modes of environmental change in the marine realm such as climate change and coastal eutrophication are multivariate phenomena. For example, increasing anthropogenic CO_2 emissions are expected to raise surface ocean pCO_2, raise mixed-layer temperatures, and lower dissolved oxygen content simultaneously

(Gruber, 2011). Similarly, increasing nutrient efflux in a coastal environment drives both declining dissolved oxygen content and substantial spikes in pCO_2 (Diaz and Rosenberg, 2008). Each of these changes requires distinct physiological adaptations on the part of marine teleosts, with different combinations of each stressor likely to lead to complex synergies or antagonisms (Pörtner et al., 2004). Yet the power of statistically derived habitat suitability indices is often limited by strong correlations between these variables (Rodda et al., 2011). Consequently, there is a high risk of overfitting the habitat space or misattributing patterns in distribution or abundance to spurious variables (Kearney et al., 2008; Rodda et al., 2011).

Similarly, the applicability of statistically derived habitat suitability indices is limited with regards to novel environmental conditions. Because such indices rely on presence/absence data, there is a lack of data on environments that are not currently extant in the field (Hirzel and Le Lay, 2008; Kearney et al., 2008). Climate change for example is a widespread forcing that is likely to push certain environmental variables such as pCO_2 beyond the levels currently seen in most habitats. Furthermore, novel combinations of variables are also difficult to predict (Kearney and Porter, 2009). The ability to explore beyond the naturally observable climate space without relying on extrapolation is a key challenge for improving the performance of habitat suitability indices.

In some cases, a physiological perspective can provide the basis for a more robust mechanistic framework (Claireaux and Lefrançois, 2007; Homyack, 2010; Kearney et al., 2008). Namely, a measured quantity known as the aerobic scope, which describes the difference between maximal aerobic metabolism and the minimum metabolic expenditure necessary for life-sustaining processes (Fry, 1971), is a promising integrative indicator of habitat quality (Lefrançois and Claireaux, 2003;

Pörtner and Knust, 2007). The magnitude of the aerobic scope in a given environment has been directly linked to the capacity for fitness-related activities such as growth, reproduction, migration, and foraging (Pörtner, 2001) both for active, highly aerobic swimmers such as pelagic piscivores for which locomotoray capacity is typically limited by aerobic scope as well as for comparatively sedentary benthic species with high anaerobic capacity for which digestive ability and burst swimming recovery are key (Clark et al., 2013). This provides the opportunity to avoid both of the two main weaknesses of correlative habitat suitability indices by basing a novel index on a factor that is directly linked to fitness under different environmental conditions. While it is possible in some cases to construct a theoretical model based on prior knowledge of a number of physiological parameters in a well-studied species (Del Raye and Weng, 2015), such models are vulnerable to modeling error due to gaps in our knowledge of physiological adaptations. Direct measurements of the output variable (aerobic scope) are a valuable opportunity to evaluate such theory-based models, and present an alternate approach to building a robust predictor of future aerobic performance.

4.1.5. Two Approaches to Constructing a Physiology-based Index

Our study uses a previously constructed data set of aerobic scope (Del Raye and Weng; in preparation) in the eurytolerant teleost *Oreochromis mossambicus* under varying oxygen, temperature, and pCO_2 conditions to fit a novel habitat suitability model. We use this fitted empirical model to evaluate a theoretical physiology-based modeling framework previously developed in Del Raye and Weng (2015). We demonstrate the use of the model in a coastal habitat within the range of *O. mossambicus*, and explore a series of future scenarios corresponding to the types of environmental perturbation that are expected to result from climate change and coastal eutrophication.

4.1.6. Methods

4.1.7. Empirical Model Construction

In creating our model we followed the example of Claireaux and Lagardére (1999) in adopting the following basic assumptions: (1) the aerobic scope follows an asymptotic curve with respect to dissolved oxygen where the asymptote (maximum aerobic scope) is attained at 100% air saturation (roughly 200 µmol O^2 kg^{-1} at 30°C), and (2) declines in oxygen concentration result in an exponential decline in aerobic scope. In addition, we assumed a similar form of the function with respect to temperature (as in Lefrançois and Claireaux (2003)) where the optimum temperature in this case is the mean water temperature of the habitat throughout the year. This assumption is consistent with the strong correlation between the mean historic temperature experienced in a habitat and the thermal optima of the aerobic scope of populations that reside there (Eliason et al., 2011; Nilsson et al., 2009). The resulting form of the model is as follows:

$$M_{scope} = \mathrm{M}_{peak} - e^{(T_{opt}-T)\lambda_T} - e^{(O_{2opt}-O_2)\lambda_O} \qquad \text{Eq. 3.}$$

where M_{scope} is the predicted aerobic scope, T and O_2 are the ambient temperature and dissolved oxygen, respectively, $\mathrm{T_{opt}}$ and $\mathrm{O_{2opt}}$ are the optimum temperature and oxygen, and λ_T and λ_O are the curvature coefficients for temperature and oxygen sensitivity. Higher λ_T or λ_O increases the sensitivity of the predicted aerobic scope to environmental conditions, narrowing the *pejus* limits (Pörtner et al., 2001) beyond which substantial declines in physiological performance begin to be seen. These model variables and symbols are as described in Table 2. While a number of studies have explored the impact of pCO_2 on aerobic performance in marine teleosts (Mckenzie et al., 2003; Melzner et al., 2009; Nilsson et al., 2009) and the interactions between pCO_2 and the cellular or molecular constituents that underlie many of the key processes driving aerobic scope have also been elucidated, this literature is still quite sparse in comparison with our understanding of temperature and oxygen effects. As a result, we chose not to explicitly model pCO_2 effects but to explore this effect purely through the empirical data.

4.1.8. Model Fitting

We trained our model on empirical measurements of aerobic scope in seawater-reared captive *O. mossambicus* under varying temperature, oxygen, and pCO_2 conditions reported previously in Del Raye and Weng (2015; in preparation). Briefly, seven measurements each of aerobic scope were conducted under temperatures of 25 and 29°C, oxygen concentrations of 100 and 200 $\mu mol\ O_2\ kg^{-1}$, and pCO_2 of 390 and 2,000 µatm, as well as various combinations of these variables. Two separate models were constructed to fit the normocapnic (pCO_2 = 390 µatm) and hypercapnic (pCO_2 = 2,000 µatm) data. Fitting was done by minimization of non-linear least squares using the Levenberg-Marquardt algorithm.

4.1.9. Theoretical Model Construction

The theoretical model followed the basic construction previously outlined in Del Raye and Weng (2015). Briefly, we created a three-box model consisting of a conceptually infinite seawater reservoir, a gill reservoir, and a reservoir for the working tissues. The maximum metabolic rate component consists of ten coupled equations drawn from classical physiological relationships (e.g., the Fick Equation, the Van't Hoff Equation, the Bohr effect) and empirical regression equations (e.g., temperature and oxygen effects on cardiac output). The estimated maximum metabolism is a function of the flux of blood between the gills and working muscles (determined by the cardiac output), and the rate of oxygen flux per unit of blood volume (determined by modeled red blood cell kinetics). The minimum metabolic rate component is based on empirical regression equations. Subtracting the minimum from maximum metabolism allows us to estimate the aerobic scope.

The models presented in Del Raye and Weng (2015) were parameterized for yellowfin tuna (*Thunnus albacares*) and a generic marine teleost using measurements of physiological parameters gleaned from the available literature. Our *O. mossambicus* theoretical model used the same approach to gather parameters specific to this species.

4.1.10. Model Evaluation

We evaluated the empirical and theoretical aerobic scope models against our measured aerobic scope dataset using the Nash-Sutcliffe model efficiency coefficient (Nash and Sutcliffe, 1970), which is an integrative measure of the correlation between the observed and predicted data as well as the degree of bias (Krause et al., 2005). Possible values for the model efficiency coefficient range from $-\infty$ to 1, with a value of zero indicating that the model output is as predictive as the mean of the observed dataset, and a value of 1 indicating perfect agreement (Krause et al., 2005). To help interpret the model efficiency values, we also calculated the coefficient of determination (r^2) as an indicator of the degree of correlation, and the percent bias.

4.1.11. Study Site

To demonstrate the use of our model we chose a location on the south shore of the island of Oahu in Hawai'i (Figure 1). *O. mossambicus* were introduced to the island in 1952 (S. L. Coles et al., 1999) and became widely distributed across multiple estuarine regions, although substantial displacement by and hybridization with the invasive blackchin tilapia (*Sarotherodon melanotheron*) has been observed in recent years (Wu and Yang, 2012). In addition to being a known habitat of *O. mossambicus* as has been confirmed by multiple surveys (S L Coles et al., 1999; United States Geological Survey, 2012; Williams and Hawaii State Department of Land and Natural Resources, 2010), our study site has also been the subject of high-frequency monitoring with moored sensors on a surface buoy since 2008 by the Pacific Islands Ocean Observing System (PacIOOS, 2015) and Sutton et al. (2014). The available record of seawater pCO_2, dissolved oxygen, temperature, and salinity spans six years with pCO_2 measurements taken every 30 minutes (with the exception of breaks in the data) and temperature, oxygen, and salinity every 20 minutes.

The proximity of the site to the Ala Wai Canal outfall (a distance of approximately 300m) underscores its potential vulnerability to anthropogenic eutrophication. The canal is an artificial tropical estuary that has historically experienced chronic problems with residential and light industrial runoff, and it is estimated that 12% of total allochthonous carbon inputs are discharged into the surrounding ocean during non-storm periods (Laws et al., 1993). Intensification of anthropogenic inputs or changes in circulation at the study site could lead to more severe problems in the future.

4.1.12. Environmental Change Scenarios

The model was evaluated using the time series of environmental conditions derived from the moored sensors in the study site. We chose four environmental change scenarios derived from the sensor data. (1) Current conditions, reflecting persistent high dissolved oxygen and low pCO_2 and including seasonally driven temperature oscillations. (2) A high CO_2 scenario with the conditions as in 1 but mean

pCO_2 raised to 2,000 µatm. (2) A high CO_2, mild hypoxia scenario with conditions as in 2 but mean dissolved oxygen lowered to 150 µmol kg^{-1}. (3) A high CO_2, moderate hypoxia scenario with conditions as in 2 but mean dissolved oxygen lowered to 100 µmol kg^{-1}. Scenarios 2-4 reflect possible future changes of the kind that could be caused by coastal eutrophication (due to factors such as nutrient runoff or increased outflow from the nearby canal) alone or in combination with climate change forcing (e.g. acidification due to the uptake of anthropogenic CO_2, hypoxia due to increased rainfall-induced runoff). A summary of the environmental change scenarios is shown in Table 1.

4.1.13. Results

4.1.14. Empirical Model Fitting

The fitted empirical model exhibited the expected behavior with distinct limiting oxygen curves characterized by changes in the maximum aerobic scope (M_{peak}) and curvature (λ_O) (Figure 2). While mean M_{peak} (maximum aerobic scope) was similar in the normocapnic and hypercapnic fit, λ_T (the temperature sensitivity coefficient) was 4-fold larger in magnitude when the model was fitted using the hypercapnic dataset, indicating a higher temperature sensitivity. Full parameter means and standard deviations are reported in Table 2

Evaluating the performance of the fitted empirical model against the observed dataset from Del Raye and Weng (2015; in preparation) revealed a Nash-Sutcliffe model efficiency coefficient of 0.71 (Figure 3). The correlation between the predicted and observed values, as indicated by the coefficient of determination (r^2), was 0.71, and the percent bias was -3.2×10^{-8}%.

4.1.15. Theoretical Model

The theoretical aerobic scope model for *O. mossambicus* was specified using physiological parameters gleaned from the literature. Due to a lack of available data, a number of cardiac parameters (e.g. λ_1, λ_2, Q_{max}) were drawn from studies performed on *Oreochromis* hybrids (*niloticus* X *mossambicus* X *hornorum*) (see note to Table 3).

Evaluating the performance of the theoretical model against the observed dataset from Del Raye and Weng (2015; in preparation) revealed a slightly lower Nash-Sutcliffe model efficiency coefficient (0.54) compared to the empirical model (Figure 4). The correlation between the predicted and observed values, as indicated by the coefficient of determination (r^2), was 0.56, and the percent bias was 0.54%.

4.1.16. The Environment of the Study Site

The six-year temperature, dissolved oxygen, and pCO_2 time series from the study site revealed a predominantly high oxygen, low pCO_2 regime with temperatures ranging seasonally from 23 to 28°C (Figure 5). Salinities were high and stable at or near 35 ppt. The oxygen record showed slight seasonal fluctuations with lower oxygen concentrations predominantly in the fall and higher concentrations in winter. One notable hypoxic event was recorded in September of 2013, with minimum dissolved oxygen concentrations reaching 51 µmol/kg^{-1}. Summary statistics are reported in Table 4.

4.1.17. Evaluating Environmental Change Scenarios

Running the fitted model on the time series of current environmental conditions in the study site (scenario 1) showed a mean predicted aerobic scope of 7.20 ± 0.90 mmol O_2 kg$^{-0.8}$ hr^{-1} (Figure 6) with seasonal temperature-driven oscillations of 0.34 ± 0.51 mmol O_2 kg$^{-0.8}$ hr^{-1} (Figure 7). Under the high pCO_2 simulation (scenario 2), mean aerobic scope fell to 6.88 ± 0.74 mmol O_2 kg$^{-0.8}$ hr^{-1}.

Seasonal oscillations also became more pronounced, with the magnitude of the oscillation reaching 0.63 ± 0.54 mmol O_2 $kg^{-0.8}$ hr^{-1}. Further declines in aerobic scope occurred under the simulated high pCO_2, mild hypoxia (scenario 3) and moderate hypoxia (scenario 4) conditions, with means of 5.95 ± 0.93 and 4.16 ± 1.56 mmol O_2 $kg^{-0.8}$ hr^{-1} respectively, and seasonally-driven oscillations of 0.86 ± 0.99 mmol O_2 $kg^{-0.8}$ hr^{-1} and 1.30 ± 1.87 mmol O_2 $kg^{-0.8}$ hr^{-1}. Aerobic scope under both scenarios 3 and 4 were significantly different from scenario 1 by $p < 0.05$.

4.1.18. Discussion

Our results demonstrate the usage of an empirically derived, physiology-based model for predicting habitat suitability in a seawater environment for the euryhaline teleost *O. mossambicus*. We describe a simple set of equations based on published relationships between temperature and oxygen on aerobic scope and fit them against a dataset of experimental measurements obtained in a controlled laboratory setting. The fitted equations are a nearly unbiased estimator of the experimental values (percentage bias = -3.2×10^{-8}%) and reproduce the following key characteristics of the measured aerobic scope:

1. Low normocapnic λ_T and high hypercapnic λ_T, leading to high temperature sensitivity only at high pCO_2. This reproduces the synergistic interaction between temperature and pCO_2 observed in the experimental data, whereby high temperatures only depressed aerobic scope at pCO_2 = 2,000μatm.
2. Similar M_{peak} at high and low pCO_2. As a result, pCO_2 has little effect as a single variable, but is important in combination with temperature due to the effect outlined above.

These characteristics have important implications when applied to the high-frequency time series of current environmental conditions and simulated environmental change, as will be discussed in subsequent sections.

4.1.19. Comparison with Theoretical Aerobic Scope Model

While the theoretical aerobic scope model also produced biologically realistic estimates of *O. mossambicus* aerobic scope, it performed less well against the experimental dataset than did the empirical model. Nash-Sutcliffe modeling efficiency coefficients indicated that while both models performed substantially better than the mean of the observed data (i.e. the case where modeling efficiency = 0), the empirical model was a closer fit (modeling efficiency = 0.71 versus 0.54), partially due to higher bias in the theoretical model (percentage bias = 0.54%). In particular, the highest aerobic scope values in the experimental dataset (aerobic scope = 9.7, 9.9, 10.5, and 10.6 mmol O_2 $kg^{-0.8}$ hr^{-1}, respectively) were poorly captured in the theoretical model.

4.1.20. Implications for O. mossambicus Under Environmental Change

The high frequency time series of environmental conditions at our study site revealed high and stable aerobic scope under current conditions (scenario 1), consistent with an area of favorable *O. mossambicus* habitat (Figure 6). Minimum aerobic scope even during the hypoxic event of 2013 never fell below 2.0 mmol O_2 $kg^{-0.8}$ hr^{-1}, and the aerobic scope at the 10th percentile was 6.9 mmol O_2 $kg^{-0.8}$ hr^{-1}. This is indicative of a eurytolerant organism that is often exposed to highly variable environmental conditions (Sardella et al., 2004). Consistent with this view, aerobic scope was not substantially affected by hypercapnia, with minimum and 10th percentile aerobic scope under scenario 2 (pCO_2 = 2,000 μatm) only slightly lower than under current conditions (1.5 and 6.5 mmol O_2 $kg^{-0.8}$ hr^{-1}, respectively).

Despite this robust environmental tolerance, the suitability of the study site as an *O. mossambicus* habitat is likely to decline substantially under our hypothetical environmental change scenarios. Scenario 3 (moderate hypoxia) caused a 1.3 mmol O_2 $kg^{-0.8}$ hr^{-1}, or 17% decline in mean aerobic scope, with minimum aerobic scope during the hypoxic event of 2013 falling below zero for a total of 3.3 hours. The environmental conditions under which aerobic scope reaches zero are termed the critical points, and indicate thresholds beyond which long-term habitation becomes impossible and animals must rely on anaerobic expenditures to survive (Pörtner et al., 2010). While *O. mossambicus* has a high anaerobic capacity (Sukumaran, 1986) and can carry out aquatic surface respiration to relieve short periods (several days) of hypoxic stress (Teichert-Coddington and Green, 1993), these conditions are likely to have strong sublethal effects. In particular, the shape of the limiting-oxygen curve (Claireaux and Lagardére, 1999) dictates that sensitivity to oxygen perturbation increases at low oxygen concentrations, and therefore even relatively small hypoxic excursions in excess of what is proposed for scenario 3 could dramatically increase the adverse outcomes. This is demonstrated by comparison with scenario 4, where mean aerobic scope was 3.1 mmol O_2 $kg^{-0.8}$ hr^{-1}, or 42% lower than under current conditions. Furthermore, environmental conditions exceeded the critical thresholds for a total of 675 data points, broken into two segments such that 35% of the points within a 41-day period, and 23% of the points within a subsequent 38-day period exceeded the critical threshold.

One of the consequences of the increased sensitivity of the model to temperature and oxygen at high pCO_2 and low oxygen, respectively, is the emergence of substantial seasonal dynamics in the higher stress scenarios. In particular, the absolute difference in mean aerobic scope between the highest month (January) and the lowest month (September) increased by 3.8-fold between scenarios 1 and 4 (Figure 7), whereas the magnitude of this seasonal fluctuation as a proportion of mean aerobic scope increased from 5% (scenario 1) to 31% (scenario 4). Temperature fluctuations in higher latitude populations are thought to drive the shift from continuous breeding as is observed in the Hawaiian population to a summer breeding season at higher latitudes (Cornish, 1998). The high degree of variability in habitat suitability indicated by the fluctuations in simulated aerobic scope could portend a similar curtailing of the breeding season under conditions analogous to scenario 4.

It is important to note that although the integrative nature of aerobic scope makes it a powerful indicator of physiological change, this same quality creates inherent uncertainties when attempting to predict specific impacts on performance or behavior (Clark et al., 2013). This is apparent when it is considered that the aerobic scope essentially describes the total available budget for aerobic energy output at a given instant. Therefore, as in any energetic budget, the impact of an overall decline in available energy on a specific activity depends upon the apportionment of energy between activities. Some populations may seek to maintain constant energy allocation for somatic growth at the expense of fluctuating gonadal growth/immune function for example, meaning that growth rates will remain unaffected even under substantial declines in aerobic scope, whereas others may seek to maintain gonadal growth at the expense of somatic growth (Lambert and Dutil, 2000; Rijnsdorp, 1990). The proportion of energy allocation can also change within populations due to biotic, environmental, or endocrine factors such as in the closely related nile tilapia (*Oreochromis niloticus*), where a bioenergetics model indicated that the proportion of aerobic metabolism allocated to biosynthesis ranged between 14–36% depending on the rate of feeding (van Dam and Pauly, 1995).

Despite these limitations, simultaneous measurements of aerobic scope and other key activities such as growth or locomotion can provide an indication of the sensitivity of specific functions to changes in aerobic scope. Unfortunately, this type of disaggregation of the total aerobic scope into its constituent activities is hampered in our study species due to the paucity of such measurements in the literature. McKenzie et al. (2003) found evidence of an association between aerobic scope and growth rate in wild type and growth hormone transgenic tilapia hybrids (*O. mossambicus* X *hornorum*), where higher growth rates in the transgenic hybrids was found to correspond with higher metabolism and aerobic scope. Similarly, Schnell and Seebacher (2008) found that critical swimming speeds in *O. mossambicus* correlated with the aerobic scope of fishes acclimated to different temperatures. Only three temperatures were tested in this study and therefore the slope of the correlation is unlikely to be reliable. However, if we assume a linear relationship between aerobic scope and critical swimming speed (as has been found in other species, e.g. Reidy et al. (2000)), this would imply a 6% reduction in critical swimming speed under scenario 2 (hypercapnia) compared to scenario 1 (control conditions), and an 18% reduction under scenario 4 (hypercapnia and moderate hypoxia).

In contrast to the paucity of published data for *O. mossambicus*, robust correlations between aerobic scope and fitness-related activities are available for other species. Such correlations cannot be taken to have predictive value for *O. mossambicus*, however, they do provide context for our results. For example, the magnitude of aerobic scope decline between scenario 1 and scenario 2 that our model predicts for *O. mossambicus*, if applied to seawater-acclimated European sea bass (*Dicentrarchus labrax*), would be sufficient to drive a 9.8% decline in specific growth (Claireaux and Lefrançois, 2007) (Table 5). In Atlantic cod (*Gadus morhua*) the same change in aerobic scope would translate to a 27% decline in growth (Claireaux et al., 2000) and a 5% decline in the maximum sustainable swimming speed (Reidy et al., 2000), while in turbot (*Scophthalmus maximus*) we would expect a 27% decline in daily feeding rate (Mallekh and Lagardére, 2002). More severe declines in aerobic scope, such as those we predict for *O. mossambicus* in scenario 4 would correspond to a 39% decline in growth rate in *D. labrax*, a 79% decline in growth rate and a 15% swimming speed decline in *G. morhua*, and an 81% feeding rate decline for *S. maximus*. These substantial impacts are indicative of a magnitude of aerobic scope reduction that would have large effects on the *O. mossambicus* population of our study area.

4.1.21. Prospects for Aerobic Scope-Based Habitat Suitability Indices

Our empirical model is a computationally cheap, physiology-based index that is well suited for use in fisheries, distribution, or end-to-end ecosystem models. Direct substitution into use cases such as Eq. 1 or 2 is made possible by dividing the modeled aerobic scope by M_{peak} to constrain the range to between 0–1. Many of the remaining limitations of our method could be addressed in future work.

One of the most significant limitations for the use of both the empirical and theoretical approaches is the large data burden required to specify the model. Climate envelope models and other statistical approaches have typically relied on abundance or presence-absence data coupled with publically available environmental data. For example, temperature and oxygen values in the World Ocean Atlas or World Ocean Database have been widely used in the marine realm (e.g. Prince and Goodyear, 2006), whereas atmospheric parameters such as mean and minimum temperature and precipitation have often been preferred in freshwater environments (e.g. Zengeya et al., 2015). By contrast, the empirical model relies on intensive swim-tunnel respirometry on live animals, and can only be carried out on species that are amenable to being kept in captivity (with rare exceptions, e.g. Farrell et al. (2003)). Similarly, the theoretical model relies upon a large volume of physiological data that has not yet been collected for all but a few exceptionally well-studied species such as Atlantic cod (*Gadus morhua*) and yellowfin and bigeye tuna (*Thunnus albacares* and *Thunnus obesus*) (see e.g. Del Raye and Weng, 2015). Creating such a physiological dataset for a poorly studied species would also require a substantial commitment of time and resources and is likely to be infeasible to carry out on more than a few species at a time. Applications such as end-to-end ecosystem models, which examine dozens of species or more, will therefore benefit by using our detailed theoretical or empirical model only on select species of particular interest. In the case of the theoretical model, prudent use of sensitivity analyses such as in Figure 8 will help to identify parameters that can be omitted or substituted with generic values without having a noticeable impact on the output values.

In cases where enough resources are available to collect more data, further refinements to the empirical model would help to buttress some important sources of uncertainty. For example, changes in the response of aerobic scope to temperature in conspecifics of different body mass have been suggested to drive population-level shifts in abundance or size structure (Pörtner et al., 2001). Conducting aerobic scope measurements across size classes could illuminate these differences and also prove important for applications such as fisheries models where the differential abundance of different size classes is extremely important (Hampton, 2000). Similarly, raising and/or breeding individuals under different environmental conditions would allow the model to take the effects of physiological plasticity and epigenetic adaptation into account. Studies on thermal acclimation of *O. mossambicus* for example have revealed substantial capacity for the adaptation of juvenile fish to chronic perturbations in temperature (Schnell and Seebacher, 2008), and the high degree of variability in the physiological input parameters for the theoretical model (e.g. H and $p50$), which gives rise to the broad distribution of predicted aerobic scope, also suggest the potential for phenotypic variability in *O. mossambicus* populations to drive future adaptation. Such additional work would go a considerable way toward improving the precision of our predictions and our confidence in our understanding of the impacts of future environmental change.

4.1.22. References

Brooks, R.P., 1997. Improving habitat suitability index models. Wildl. Soc. Bull. 25, 163–167.

Buthelezi, P., Wepener, V., Cyrus, D., 2000. The sublethal effects of zinc at different water temperatures on selected haematological variables in *Oreochromis mossambicus*. African J. Aquat. Sci. 25, 146–151. doi:10.2989/160859100780177659

Claireaux, G., Lagardére, J.P., 1999. Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. J. Sea Res. 42, 157–168. doi:10.1016/S1385-1101(99)00019-2

Claireaux, G., Lefrançois, C., 2007. Linking environmental variability and fish performance: integration through the concept of scope for activity. Philos. Trans. R. Soc. B 362, 2031–2041. doi:10.1098/rstb.2007.2099

Claireaux, G., Webber, D.M., Lagardére, J.P., Kerr, S.R., 2000. Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (Gadus morhua). J. Sea Res. 44, 257–265. doi:10.1016/S1385-1101(00)00053-8

Clark, T.D., Sandblom, E., Jutfelt, F., 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. J. Exp. Biol. 216, 2771–2782. doi:10.1242/jeb.084251

Coles, S.L., DeFelice, R., Eldredge, L.G., 1999. Nonindigenous Marine Species Introductions in the Harbors of the South and West Shores of Oahu, Hawaii, Bishop Museum Technical Report. Honolulu, HI.

Coles, S.L., DeFelice, R.C., Eldredge, L.G., Carlton, J.T., 1999. Historical and recent introductions of non-indigenous marine species into Pearl Harbor, Oahu, Hawaiian Islands. Mar. Biol. 135, 147–158. doi:10.1007/s002270050612

Cornish, D.A., 1998. Seasonal steroid hormone profiles in plasma and gonads of the tilapia, Oreochromis mossambicus. Water SA 24, 257–263.

Del Raye, G., Weng, K.C., 2015. An aerobic scope-based habitat suitability index for predicting the effects of multi-dimensional climate change stressors on marine teleosts. Deep. Res. II 113, 280–290. doi:10.1016/j.dsr2.2015.01.014

Diaz, R.J., Rosenberg, R., 2008. Spreading dead zones and consequences for marine ecosystems. Science (80-.). 321, 926–929. doi:10.1126/science.1156401

Eliason, E.J., Clark, T.D., Hague, M.J., Hanson, L.M., Gallagher, Z.S., Jeffries, K.M., Gale, M.K., Patterson, D. a., Hinch, S.G., Farrell, a. P., 2011. Differences in Thermal Tolerance Among Sockeye Salmon Populations. Science (80-.). 332, 109–112. doi:10.1126/science.1199158

Farrell, a. P., Lee, C.G., Tierney, K., Hodaly, a., Clutterham, S., Healey, M., Hinch, S., Lotto, a., 2003. Field-based measurements of oxygen uptake and swimming performance with adult Pacific salmon using a mobile respirometer swim tunnel. J. Fish Biol. 62, 64–84. doi:10.1046/j.1095-8649.2003.00010.x

Fry, F.E.J., 1971. The Effect of Environmental Factors on the Physiology of Fish, in: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology. Academic Press, New York, pp. 1–98.

Gruber, N., 2011. Warming up, turning sour, losing breath: ocean biogeochemistry under global change. Philos. Trans. R. Soc. A 369, 1980–1996. doi:10.1098/rsta.2011.0003

Guisan, a, Zimmermann, N.E., 2000. Predictive habitat distribution models in ecology. Ecol. Modell. 135, 147–186. doi:10.1016/S0304-3800(00)00354-9

Hampton, J., 2000. Natural mortality rates in tropical tunas: size really does matter. Can. J. Fish. Aquat. Sci. 57, 1002–1010. doi:10.1139/f99-287

Hirzel, a. H., Helfer, V., Metral, F., 2001. Assessing habitat-suitability models with a virtual species. Ecol. Modell. 145, 111–121. doi:10.1016/S0304-3800(01)00396-9

Hirzel, A.H., Le Lay, G., 2008. Habitat suitability modelling and niche theory. J. Appl. Ecol. 45, 1372–1381. doi:10.1111/j.1365-2664.2008.01524.x

Hirzel, A.H., Le Lay, G., Helfer, V., Randin, C., Guisan, A., 2006. Evaluating the ability of habitat suitability models to predict species presences. Ecol. Modell. 199, 142–152. doi:10.1016/j.ecolmodel.2006.05.017

Homyack, J. a., 2010. Evaluating habitat quality of vertebrates using conservation physiology tools. Wildl. Res. 37, 332. doi:10.1071/WR08093

Kearney, M., Phillips, B.L., Tracy, C.R., Christian, K.A., Betts, G., Porter, W.P., 2008. Modelling species distributions without using species distributions: the cane toad in Australia under current and future climates. Ecography (Cop.). 31, 423–434. doi:10.1111/j.2008.0906-7590-05457.x

Kearney, M., Porter, W., 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. Ecol. Lett. 12, 1–17.

Krause, P., Boyle, D.P., Bäse, F., 2005. Comparison of different efficiency criteria for hydrological model assessment. Adv. Geosci. 5, 89–97. doi:10.5194/adgeo-5-89-2005

Lague, S.L., Speers-Roesch, B., Richards, J.G., Farrell, A.P., 2012. Exceptional cardiac anoxia tolerance in tilapia (Oreochromis hybrid). J. Exp. Biol. 215, 1354–65. doi:10.1242/jeb.063362

Lambert, Y., Dutil, J.-D., 2000. Energetic consequences of reproduction in Atlantic cod (Gadus morhua) in relation to spawning level of somatic energy reserves. Can. J. Fish. Aquat. Sci. 57, 815–825. doi:10.1139/f00-022

Laws, E.A., Doliente, D., Hiayama, J., Hokama, M.-L., Kim, K., Li, D., Minami, S., Morales, C., 1993. Hypereutrophication of the Ala Wai Canal, Oahu, Hawaii: Prospects for cleanup. Pacific Sci. 47, 59–75.

Lefrançois, C., Claireaux, G., 2003. Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole Solea solea. Mar. Ecol. Prog. Ser. 259, 273–284.

Lehodey, P., Senina, I., Murtugudde, R., 2008. A spatial ecosystem and populations dynamics model (SEAPODYM) – Modeling of tuna and tuna-like populations. Prog. Oceanogr. 78, 304–318. doi:10.1016/j.pocean.2008.06.004

Mallekh, R., Lagardére, J.P., 2002. Effect of temperature and dissolved oxygen concentration on the metabolic rate of the turbot and the relationship between metabolic scope and feeding demand. J. Fish Biol. 60, 1105–1115. doi:10.1006/jfbi.2002.1918

Maunder, M.N., Hinton, M.G., Bigelow, K.A., Langley, A.D., 2006. Developing Indices of Abundance Using Habitat Data in a Statistical Framework. Bull. Mar. Sci. 79, 545–559.

McKenzie, D.J., Martinez, R., Morales, A., Acosta, J., Morales, R., Taylor, E.W., Steffensen, J.F., Estrada, M.P., 2003. Effects of growth hormone transgenesis on metabolic rate, exercise performance and hypoxia tolerance in tilapia hybrids. J. Fish Biol. 63, 398–409. doi:10.1046/j.1095-8649.2003.00162.x

Mckenzie, D.J., Piccolella, M., Valle, A.Z.D., Taylor, E.W., Bolis, C.L., Steffensen, J.F., 2003. Tolerance of chronic hypercapnia by the European eel Anguilla anguilla. J. Exp. Biol. 206, 1717–1726. doi:10.1242/jeb.00352

Melzner, F., Göbel, S., Langenbuch, M., Gutowska, M.A., Pörtner, H.-O., Lucassen, M., 2009. Swimming performance in Atlantic Cod (Gadus morhua) following long-term (4-12 months) acclimation to elevated seawater P(CO2). Aquat. Toxicol. 92, 30–7. doi:10.1016/j.aquatox.2008.12.011

Nash, J.E., Sutcliffe, J. V, 1970. River Flow Forecasting Through Conceptual Models Part I—A Discussion of Principles. J. Hydrol. 10, 282–290. doi:10.1016/0022-1694(70)90255-6

Nilsson, G.E., Crawley, N., Lunde, I.G., Munday, P.L., 2009. Elevated temperature reduces the respiratory scope of coral reef fishes. Glob. Chang. Biol. 15, 1405–1412. doi:10.1111/j.1365-2486.2008.01767.x

PacIOOS, 2015. PacIOOS Water Quality Buoy AW (WQB-AW): Ala Wai, Oahu, Hawaii.

Perez, J.E., Maclean, N., 1976. The haemoglobins of the fish Sarotherodon mossambicus (Peters): functional significance and ontogenetic changes. J. Fish Biol. 9, 447–455.

Peterson, A., 2006. Uses and requirements of ecological niche models and related distributional models. Biodivers. Informatics 3, 59–72. doi:10.1182/blood-2009-09-244962.An

Pörtner, H.-O., 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88, 137–146. doi:10.1007/s001140100216

Pörtner, H.-O., Berdal, B., Blust, R., Brix, O., Colosimo, A., De Wachter, B., Giuliani, A., Johansen, T., Fischer, T., Knust, R., 2001. Climate induced temperature effects on growth performance, fecundity and recruitment in marine fish: developing a hypothesis for cause and effect relationships in Atlantic cod (Gadus morhua) and common eelpout (Zoarces viviparus). Cont. Shelf Res. 21, 1975–1997. doi:10.1016/S0278-4343(01)00038-3

Pörtner, H.-O., Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science (80-.). 315, 95–7. doi:10.1126/science.1135471

Pörtner, H.O., Mark, F.C., Bock, C., 2004. Oxygen limited thermal tolerance in fish? Answers obtained by nuclear magnetic resonance techniques. Respir. Physiol. Neurobiol. 141, 243–260. doi:10.1016/j.resp.2004.03.011

Pörtner, H.O., Schulte, P.M., Wood, C.M., Schiemer, F., 2010. Niche dimensions in fishes: an integrative view. Physiol. Biochem. Zool. 83, 808–26. doi:10.1086/655977

Prince, E.D., Goodyear, C.P., 2006. Hypoxia-based habitat compression of tropical pelagic fishes. Fish. Oceanogr. 15, 451–464. doi:10.1111/j.1365-2419.2005.00393.x

Reidy, S.P., Kerr, S.R., Nelson, J.A., 2000. Aerobic and anaerobic swimming performance of individual Atlantic cod. J. Exp. Biol. 203, 347–57.

Rijnsdorp, A.D., 1990. The mechanism of energy allocation over reproduction and somatic growth in female North Sea plaice, Pleuronectes platessa L. Netherlands J. Sea Res. 25, 279–289. doi:10.1016/0077-7579(90)90027-E

Rodda, G.H., Jarnevich, C.S., Reed, R.N., 2011. Challenges in identifying sites climatically matched to the native ranges of animal invaders. PLoS One 6. doi:10.1371/journal.pone.0014670

Sardella, B.A., Cooper, J., Gonzalez, R.J., Brauner, C.J., 2004. The effect of temperature on juvenile Mozambique tilapia hybrids (Oreochromis mossambicus x O. urolepis hornorum) exposed to full-strength and hypersaline seawater. Comp. Biochem. Physiol. Part A 137, 621–629. doi:10.1016/j.cbpb.2003.12.003

Schmittner, A., Oschlies, A., Matthews, H.D., Galbraith, E.D., 2008. Future changes in climate, ocean circulation, ecosystems, and biogeochemical cycling simulated for a business-as-usual CO 2 emission scenario until year 4000 AD. Global Biogeochem. Cycles 22, 1–21. doi:10.1029/2007GB002953

Schnell, A.K., Seebacher, F., 2008. Can phenotypic plasticity facilitate the geographic expansion of the tilapia Oreochromis mossambicus? Physiol. Biochem. Zool. 81, 733–742. doi:10.1086/592027

Sukumaran, N., 1986. Oxygen consumption, carbon dioxide output and nitrogen excretion in cichlid fish, Oreochromis mossambicus (Peters), with special reference to swimming speed. Proc. Indian Acad. Sci. (Anim. Sci.) 95, 215–222.

Sutton, A., Sabine, C., Maenner, S., Musielewicz, S., Bott, R., Osborne, J., 2014. High resolution ocean and atmosphere pCO2 time-series measurements from mooring Alawai_158W_21N. doi:10.3334/CDIAC/OTG.TSM_ALAWAI_158W_21N

Teichert-Coddington, D., Green, B.W., 1993. Tilapia yield improvement through maintenance of minimal oxygen concentrations in experimental grow-out ponds in Honduras. Aquaculture 118, 63–71.

Thuiller, W., 2004. Patterns and uncertainties of species' range shifts under climate change. Glob. Chang. Biol. 10, 2020–2027. doi:10.1111/j.1365-2486.2004.00859.x

Travers, M., Shin, Y.-J., Jennings, S., Cury, P., 2007. Towards end-to-end models for investigating the effects of climate and fishing in marine ecosystems. Prog. Oceanogr. 75, 751–770. doi:10.1016/j.pocean.2007.08.001

United States Geological Survey, 2012. United States Geological Survey Nonindigenous Aquatic Species Database.

van Dam, A.A., Pauly, D., 1995. Simulation of the effects of oxygen on food consumption and growth of Nile tilapia, Oreochromis niloticus (L.). Aquac. Res. 26, 427–440.

Waarde, B.Y.A.V.A.N., Dijk, P.V.A.N., Den, G.V.A.N., Bonga, W., Addink, A., 1990. P-NMR Studies on Acid-Base Balance and Energy Metabolism of Acid-Exposed Fish. J. Exp. Biol. 154, 223–236.

Williams, I., Hawaii State Department of Land and Natural Resources, 2010. Fish stock surveys from 41 sites on the islands of Oahu and Hawaii from September 11, 1952 to December 28, 2000 (NODC Accession 0002754).

Wu, L., Yang, J., 2012. Identifications of Captive and Wild Tilapia Species Existing in Hawaii by Mitochondrial DNA Control Region Sequence. PLoS One 7, 1–9. doi:10.1371/journal.pone.0051731

Wu, S.M., Deng, a. N., 2006. Effect of cadmium on hematological functions in tilapia (Oreochromis mossambicus). Bull. Environ. Contam. Toxicol. 76, 891–898. doi:10.1007/s00128-006-1002-2

Yamamoto, K.-I., 1992. Relationship of respiration to body weight in the tilapia Oreochromis niloticus under resting and normoxic conditions. Comp. Biochem. Physiol. - A Physiol. 103, 81–83.

Zengeya, T., Booth, A., Chimimba, C., 2015. Broad Niche Overlap between Invasive Nile Tilapia Oreochromis niloticus and Indigenous Congenerics in Southern Africa: Should We be Concerned? Entropy 17, 4959–4973. doi:10.3390/e17074959

4.1.23. Figures and Tables

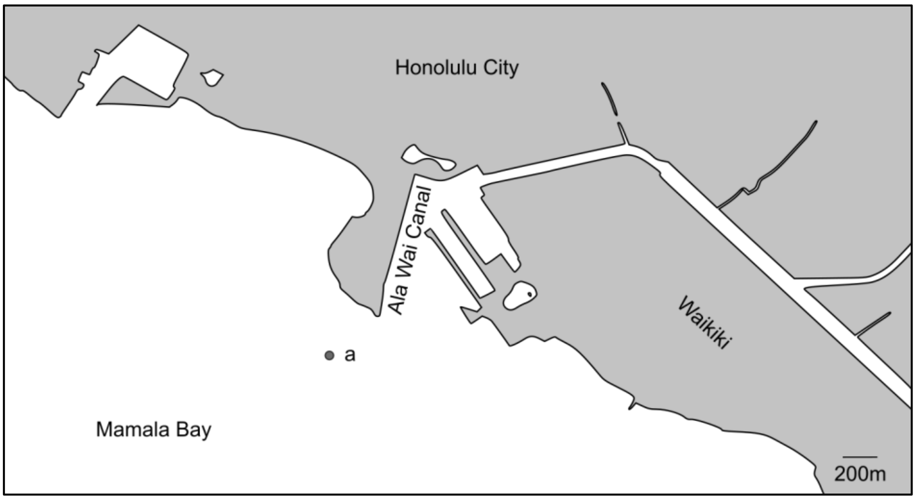

Figure 1: Map of study site (a) located at 21.280°N, 157.848°W, showing the proximity to the Ala Wai Canal outfall. Water depth at point a = 7.6 m. Map data from Google (2015).

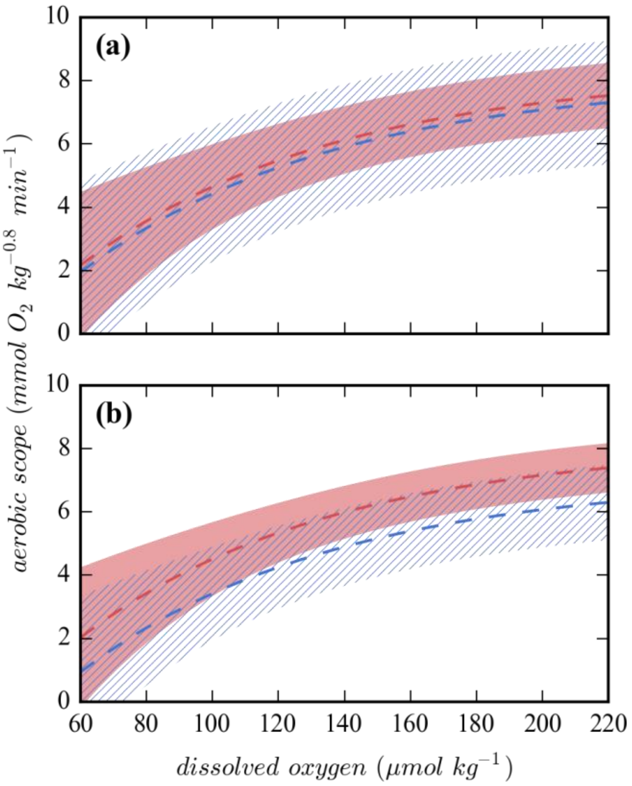

Figure 2: Demonstration of empirical model aerobic scope model for *Oreochromis mossambicus*. **(a)** Expected aerobic scope under normocapnic conditions (pCO_2 = 390

µatm) at 25°C (red dotted line) and 29°C (blue dotted line). The red shaded and blue hatched regions indicate the 95% confidence intervals of the fitted model at 25°C and 29°C, respectively. **(b)** Mean aerobic scope under hypercapnic conditions (pCO_2 = 2,000 µatm) at 25°C (red dotted line) and 29°C (blue dotted line). Color and hatching of the 95% confidence are as in **(a)**.

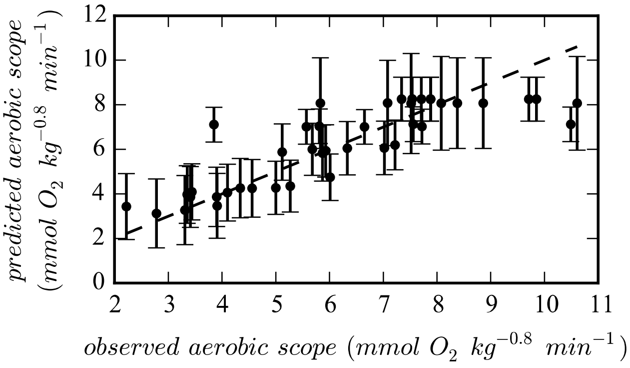

Figure 3: Observed versus predicted aerobic scope for *Oreochromis mossambicus* using an empirical aerobic scope model. Error bars indicate the 95% confidence interval of the fitted model. The dotted line shows the 1:1 line of perfect agreement. Nash-Sutcliffe model efficiency coefficient = 0.71, r^2 = 0.71, and absolute percent bias < 0.01%. N = 42 points.

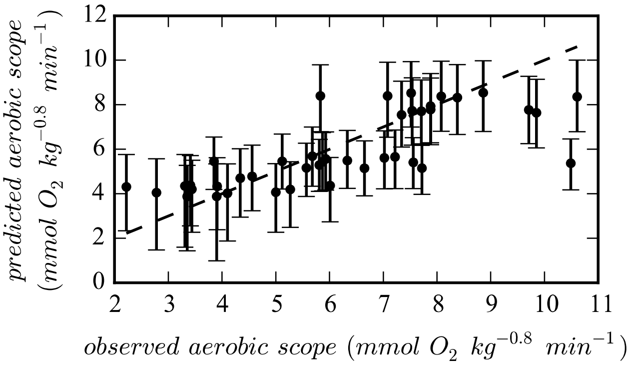

Figure 4: Observed versus predicted aerobic scope for *Oreochromis mossambicus* using a theoretical aerobic scope model. Error bars indicate 95% confidence intervals,

where the magnitude of the uncertainty is a product of the observed variability in input physiological parameters. The dotted line shows the 1:1 line of perfect agreement. Nash-Sutcliffe model efficiency coefficient = 0.54, r^2 = 0.56, and absolute percent bias = 0.54%. N = 42 points.

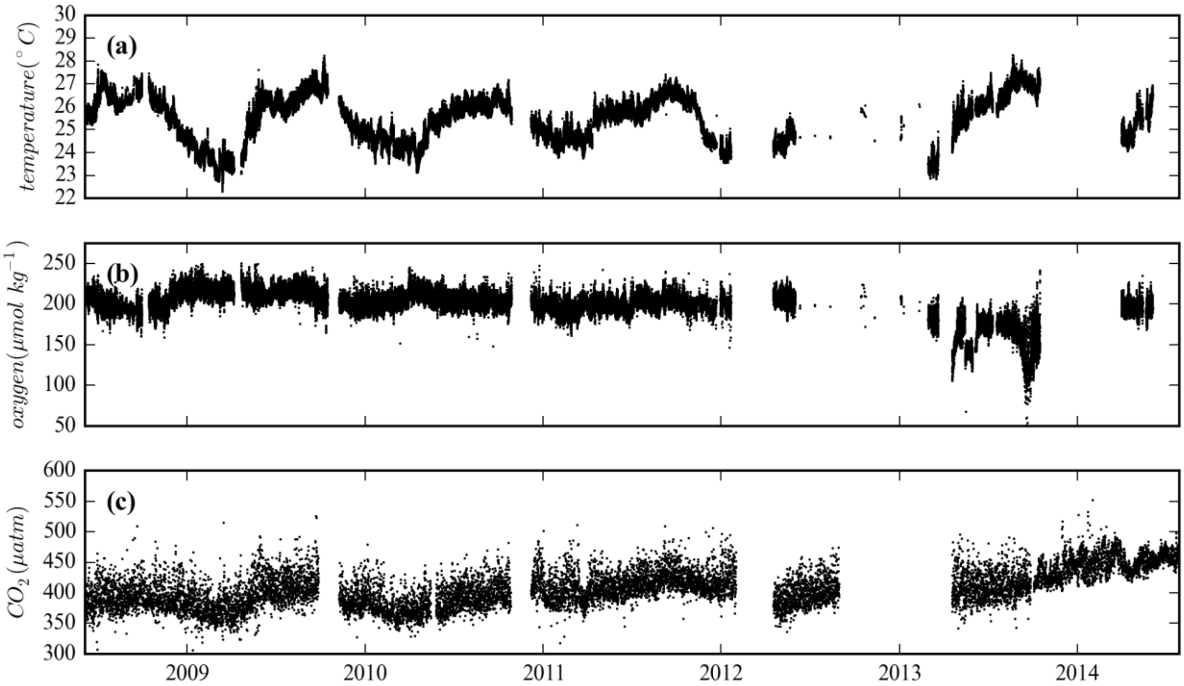

Figure 5: Time series of observed environmental parameters at the study site. The number of temperature **(a)** and oxygen **(b)** records shown is 98,568. The number of pCO_2 **(c)** records is 14,670. Data from Sutton et al. (2014) and the Pacific Islands Ocean Observing System (PacIOOS, 2015)

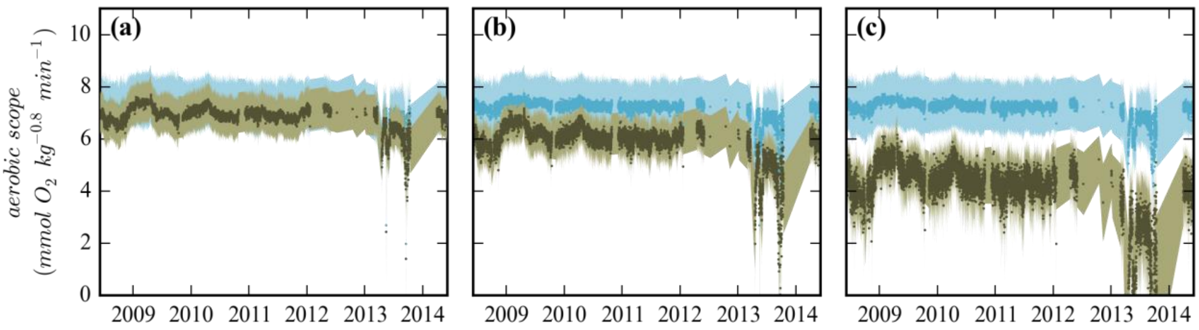

Figure 6: Simulated aerobic scope under four environmental change scenarios. Current conditions (scenario 1, blue) versus future conditions (green) under **(a)** simulated hypercapnia (scenario 2), **(b)** simulated hypercapnia and mild hypoxia (scenario 3), and **(c)** simulated hypercapnia with moderate hypoxia (scenario 4). See Table 1 for a summary of the environmental change scenarios. Lines show mean values and shaded regions indicate 95% confidence.

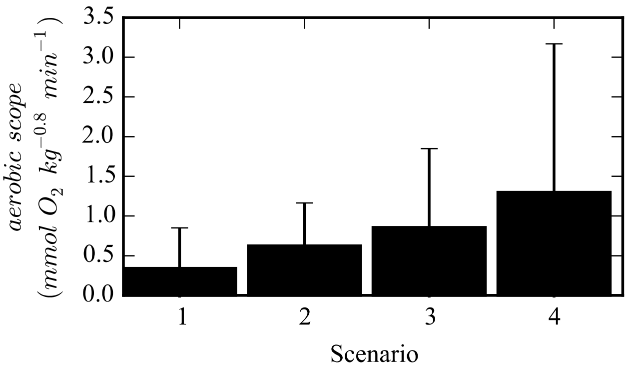

Figure 7: Emergence of seasonal dynamics under high stress environmental change scenarios. The difference in simulated aerobic scope between the peak (January) and lowest (September) months are shown for each environmental change scenario. Black bars indicate the mean difference across n = 6 years. Error bars show one standard deviation.

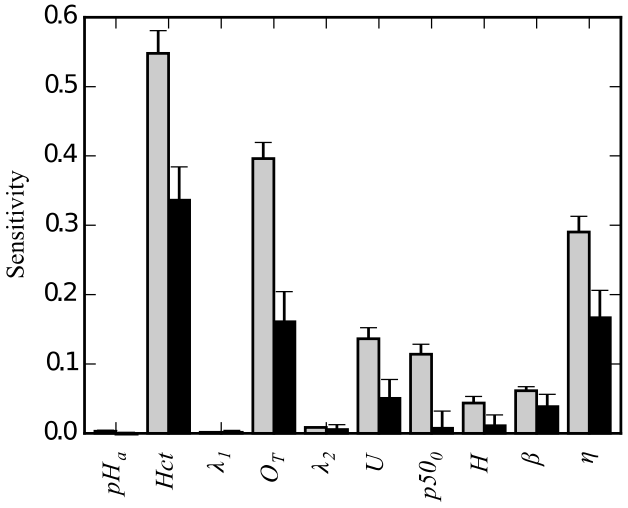

Figure 8: Sensitivity of the theoretical aerobic scope model to selected input parameters. Bars show the magnitude of Monte Carlo estimates of Sobol sensitivity indices. First order main effects (black bars) indicate the effect of uncertainty in the given parameter on the uncertainty of the estimated aerobic scope. Total effects (grey bars) show the sum of the main effects plus interactions between the given parameter and all other parameters. Error bars indicate one standard deviation. Parameter labels correspond to those shown in Table 3.

Table 1: Summary of environmental change scenarios.

Scenario	**Description**	**Temperature range (°C)**	**Mean [O_2] (µmol kg^{-1})**	**Mean pCO_2 (µatm)**
1	Present-day	22.3–28.3	200	407
2	Hypercapnic	22.3–28.3	200	2,000
3	Mild hypoxia	22.3–28.3	100	2,000
4	Severe hypoxia	22.3–28.3	50	2,000

Table 2: Fitting parameters for aerobic scope model under normocapnic (pCO_2 = 400 µatm) and hypercapnic (pCO_2 = 2000 µatm) conditions.

Symbol	**Parameter**	**Units**	**Mean (sd) for Normocapnia**	**Mean (sd) for Hypercapnia**
M_{peak}	Peak aerobic scope	mmol O_2 kg$^{-0.8}$ hr^{-1}	9.26 (0.50)	9.01 (0.39)
λ_T	Temperature sensitivity coefficient	*n/a*	-0.042 (0.143)	-0.165 (0.056)
λ_O	Oxygen sensitivity coefficient	*n/a*	0.013 (0.001)	0.013 (0.001)

Table 3: List of input parameters for theoretical aerobic scope model. Values given without standard deviations were treated as constants. Data from Lague et al. (2012) (marked with an asterisk) was collected using a tilapia hybrid (*Oreochromis niloticus* x *mossambicus* x *hornorum*).

Parameter (units)	**Symbol**	**Mean (sd)**	**Reference**
Reference temperature (K)	T_0	293.15	Perez and Maclean, 1976
Reference pH (K)	pH_0	7.5	Perez and Maclean, 1976
Mean cell hemoglobin concentration (g Hb/L)	$\overline{H}$	1000	Buthelezi et al., 2000
Reference cardiac output (L blood/kg body	Q_{max}	6.3x10^{-4}	Lague et al., 2012*

sec K)			
Minimum venous pO2 (µatm)	pO_{2v}	3950	Wu and Deng, 2006
Arterial pH	pH_a	7.78 (0.06)	Waarde et al., 1990
Hematocrit (volume fraction)	Hct	0.50 (0.05)	Buthelezi et al., 2000
Cardiac pO$_2$ sensitivity (L blood/kg body sec µatm O$_2$)	λ_1	3300 (470)	Lague et al., 2012*
Cardiac pO$_2$ threshold (µatm)	O_T	$8.1\text{x}10^{-5}$ ($2.7\text{ x}10^{-5}$)	Lague et al., 2012*
Cardiac temperature threshold (K)	T_T	303.15 (12.38)	Schnell and Seebacher, 2008
Cardiac temperature sensitivity (L blood/kg body sec K)	λ_2	0.05 (0.009)	Schnell and Seebacher, 2008
Oxygen utilization (partial pressure fraction)	U	0.874 (0.303)	Yamamoto, 1992
Hemoglobin half-saturation constant (µatm)	$p50_0$	17600 (2800)	Perez and Maclean, 1976
Hemoglobin heat of oxygenation (kcal/mole)	H	-27.2 (9.4)	Perez and Maclean, 1976
Extracellular Bohr coefficient (Δlog p50/ ΔpH)	β	-0.16 (0.05)	Perez and Maclean, 1976
Hill coefficient	η	2.1 (0.4)	Perez and Maclean, 1976

Table 4: Observed environmental conditions in the study site between June 2008 and July 2014. N = 98,568 for temperature, salinity, and oxygen. N = 14,670 for pCO$_2$.

Data from Sutton et al. (2014) and the Pacific Islands Ocean Observing System (PacIOOS, 2015).

Parameter (units)	**Mean (sd)**
Temperature (°C)	25.4 (1.0)
Salinity (ppt)	34.9 (0.3)
Oxygen (µmol kg^{-1})	199.6 (17.2)
pCO_2 (µatm)	407.7 (30.3)

Table 5: Predicted change in aerobic scope (M_{scope}) of *Oreochromis mossambicus* under scenarios 2-4 compared to the control scenario (scenario 1), and comparisons to the expected magnitude of change in fitness-related activities (specific growth rate (*SGR*), critical swimming speed (U_{crit}), and daily feeding rate (*DFR*)) for three different species under analogous aerobic scope declines. Columns 4-7 were calculated based on data from Claireaux and Lefrançois (2007), Claireaux et al. (2000), Mallekh and Lagardére (2002), and Reidy et al. (2000).

Scenario	ΔM_{scope} **(mmol O_2 kg$^{-0.8}$ hr^{-1})**	ΔM_{scope} **(%)**	ΔSGR **(*Dicentrarchus labrax*; %)**	ΔSGR **(*Gadus morhua*; %)**	ΔU_{crit} **(*Gadus morhua*; %)**	ΔDFR **(*Scophthalmus maximus*; %)**
2	-1.31	-16	-9.8	-27	-5	-27
3	-2.22	-27	-19.5	-43	-9	-46
4	-3.96	-48	-39.0	-79	-16	-81

5. Summary and Conclusions

5.1. Summary

This thesis demonstrated three methods of forecasting the impact of climate-change driven environmental change on marine teleosts using a mechanistic, physiological framework—theoretical modeling (Chapter 2), direct experimentation (Chapter 3), and empirical models (Chapter 4). Overall, all three methods indicate that a combination of stressors, namely CO_2-induced ocean acidification, surface warming, and deoxygenation, are likely to interact in complex ways to impair aerobic performance in marine teleosts.

In Chapter 2 for example, we showed that yellowfin tuna (*Thunnus albacares*) are likely to experience pronounced vertical habitat compression in Eastern Tropical Pacific due to a combination of high temperature, deoxygenation in the oxygen minimum layer, and high pCO_2. This shoaling of the vertical habitat is particularly important because it is likely to increase the catchability of *T. albacares* in this region by bringing the tuna closer to surface-based fishing gear and also by decreasing the total habitat volume. Without correcting for this change in catchability, future managers run the risk of overestimating the stock of tuna in the region and consequently allowing the population to become overfished. Incorporating our work into end-to-end ecosystem models of the type exemplified by SEAPODYM and APECOSM (Lehodey et al., 2008; Maury, 2010) would also allow researchers to explore the ramifications of this vertical habitat compression on the interaction between comparatively surface-bound *T. albacares* and forage species that reside in the deeper oxygen minimum layer. The potential implications of such a study include forecasting a shift in the competitive advantage between tunas, whose ability to make forays into the oxygen minimum zone depends partly on drawing oxygen from the surrounding water, and marine mammals such as cetaceans and elephant seals, which are only constrained by the distance of travel. To our knowledge, no work examining such a trend has been carried out to date.

In Chapter 3, we showed that deoxygenation and hypercapnia interact synergistically with high temperature to impair aerobic scope in Mozambique tilapia (*Oreochromis mossambicus*). This is an important result because while previous research had strongly suggested that the tolerance of this species to temperature, oxygen, and pH stress would insulate it against future change (Costa-Pierce and Doyle, 1997; Krishna Murthy et al., 1984; Rappaport et al., 1976), we showed that even though aerobic performance is unaffected by single-stressor perturbations, multiple simultaneous stressors had a significant effect. While *O. mossambicus* is a coastal species that is unlikely to experience the kinds of open-water oxygen minimum zone expansion that we explored in *T. albacares*, there is still a strong possibility that coastal hypoxia driven by anthropogenic eutrophication combined with climate-change induced water column stratification could expose it to increasingly severe hypoxia, hypercapnia, and hyperthermia (Cai et al., 2011; Diaz and Rosenberg, 2008; Melzner et al., 2012). Furthermore, our results provide an indication that relatively stenotolerant species that are not already pre-adapted to declines in water quality could suffer more drastic constraints on their aerobic capacity. Comparisons of our results against future studies on a variety of species would help to elucidate this difference. A particularly fascinating comparison would be between our study species, *O. mossambicus* and the variety of tilapia hybrids that have been developed for saltwater aquaculture, enabling us to explore any links between aerobic performance (i.e. active versus sedentary species) or tolerance to single-stressor perturbations on climate change resilience.

In Chapter 4 we developed a novel form of an empirical model that combined a widely used framework for understanding temperature effects on aerobic scope (oxygen limited thermal tolerance (Pörtner, 2002)) and a similar framework for oxygen effects (limiting oxygen continuum (Claireaux and Lagardére, 1999)). By fitting the model to the experimental results obtained in Chapter 3 and inputting high-frequency observations of the environmental conditions in an actual *O. mossambicus* habitat, we were able to project the ways in which aerobic scope could be affected under a variety of possible environmental change scenarios. Our results indicate a large aerobic scope decline that is likely to result in substantial reductions in the capacity for growth, feeding, or locomotory ability. Unfortunately, due to a lack of an equivalently well-developed theory for understanding the effects of hypercapnia on aerobic scope, we were unable to incorporate a pCO_2 response in this model. Comparisons of aerobic performance across a larger number of hypercapnic treatments would help to identify a plausible form for such an aerobic scope versus pCO_2 curve, and is a promising area of study for the future. Because *O. mossambicus* is a euryhaline species that is capable of robust growth both in seawater and freshwater, comparisons of the pCO_2 response across a salinity gradient would be especially interesting because the difference in the availability of carbonate ions in seawater versus freshwater could reveal important mechanistic differences in the physiological response (Perry and Gilmour, 2006).

5.2. Suggestions for Future Work

Going forward, more complex experimental techniques could lead to an expanded understanding of the sub-organism level physiological processes underpinning the patterns that we revealed in this thesis. For example, in vivo magnetic resonance imaging (MRI) and nuclear magnetic resonance spectroscopy (NMR) are two methods that have been successfully demonstrated in un-anaesthetized marine teleosts but have only been rarely used to date (Pörtner et al., 2010; Van der Linden et al., 2004). ^{31}P-NMR in particular can be performed on fish undergoing exercise in a swim-tunnel respirometer of the kind we utilized in this thesis (Bock et al., 2007), making it possible to continuously monitor intracellular pH and the turnover of high-energy phosphates such as ATP and phosphocreatine in fishes at both standard and active metabolic rates. Complementary measurements of blood flow and tissue oxygenation in restrained, sedentary fishes can be monitored using MRI (Mark et al., 2002). While the temporal resolution of MRI precludes its use on exercising fishes, the phenomena of excess post-exercise metabolism (Lee et al., 2003) or, alternatively, specific dynamic action (Fitzgibbon et al., 2007) could be used to monitor restrained animals with heightened metabolic rates due to previous exercise or food consumption, respectively. The combination of cardiac performance, tissue oxygenation, and pH status would provide powerful new insights into many of the constituent processes that determine the overall metabolic rate under conditions of aerobic stress. Such insights could be instrumental in understanding broader inter-species trends including the possible effects of climate change on species interactions. For example, an MRI analysis of *in vivo* physiology could be used to isolate a limiting factor for maintaining high aerobic scope under a given set of environmental challenges. In the hypothetical case that oxygen delivery to the cardiac muscles was found to limit aerobic scope among high-energy pelagic predators for example, this could be used to generate the prediction that tunas with highly developed coronary arteries (e.g. bluefin tunas) would be more resilient to the given set of environmental changes than competing pelagic predators with no coronary vasculature, and therefore may gain a competitive advantage.

An equally intriguing avenue for future work is the extension of metabolic rate measurements into the field. While there have been some attempts to perform respirometry in the wild (Farrell et al., 2003), the most versatile methods involve the use of metabolic rate proxies such as heart rate and overall dynamic body acceleration (ODBA) (Lucas et al., 1993; Shepard et al., 2008). Heart-rate monitoring tags affixed to free-swimming fish provide an indicator of metabolic rate via the Fick equation, which relates cardiac output (a product of heart rate and stroke volume) to metabolism (Brodeur, 2001). While many teleosts are able to modify stroke volume to an equivalent or larger degree than heart rate (Farrell, 1996), repeated studies have shown that heart rate can be a good indicator of metabolism (Clark et al., 2010), as well as of temperature stress (Heath and Hughes, 1973) and poor water quality (Milligan and Wood, 1982) in many species. Similarly, strong correlations have been demonstrated between ODBA and field metabolic rate (Halsey et al., 2009). ODBA is an especially interesting index of metabolic activity because, rather than reflecting a physiological component of overall metabolism, it reflects a functional component – namely the metabolic cost of movement (Gleiss et al., 2011). Although this has mainly been treated in the literature as being equivalent to overall metabolic rate in ectothermic animals where the locomotory component accounts for the vast majority of the total metabolic expenditure (Lucas et al., 1993), an alternative interpretation would be to treat it as analogous to the scope for growth concept (Widdows et al., 1997), which attempts to isolate the investment of metabolic energy into a specific activity – namely somatic growth. Used together, a general metabolic rate indicator (heart rate) and a proxy for the locomotory component (ODBA) could be examined across natural gradients in temperature, oxygen, or pCO_2 to determine how maximum and minimum metabolic rates are affected by these parameters in the wild. Examining stable environmental gradients in the field is an important method of accounting for trans-generational adaptation of the type that is likely to accompany future climate change perturbations (Johnson et al., 2012). While the technology for doing this exists, few studies appear to be taking advantage of it to produce this valuable counterpoint to laboratory experiments.

5.3. List of References

Bock, C., Lurman, G.J., Wittig, R., Webber, D.M., Pörtner, H.-O., 2007. Muscle Bioenergetics of Speeding Fish: In Vivo MRS Studies in a 4.7 T Magnetic Resonance Scanner with an Integrated Swim Tunnel. Concepts Magn. Reson. Part B 1–12. doi:10.1002/cmr.b

Brodeur, J., 2001. Assessment of cardiac output as a predictor of metabolic rate in rainbow trout. J. Fish Biol. 58, 439–452. doi:10.1006/jfbi.2000.1461

Cai, W.-J., Hu, X., Huang, W.-J., Murrell, M.C., Lehrter, J.C., Lohrenz, S.E., Chou, W.-C., Zhai, W., Hollibaugh, J.T., Wang, Y., Zhao, P., Guo, X., Gundersen, K., Dai, M., Gong, G.-C., 2011. Acidification of subsurface coastal waters enhanced by eutrophication. Nat. Geosci. 4, 766–770. doi:10.1038/ngeo1297

Claireaux, G., Lagardére, J.P., 1999. Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. J. Sea Res. 42, 157–168. doi:10.1016/S1385-1101(99)00019-2

Clark, T.D., Sandblom, E., Hinch, S.G., Patterson, D. a., Frappell, P.B., Farrell, a. P., 2010. Simultaneous biologging of heart rate and acceleration, and their relationships with energy expenditure in free-swimming sockeye salmon (Oncorhynchus nerka). J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 180, 673–684. doi:10.1007/s00360-009-0442-5

Costa-Pierce, B.A., Doyle, R.W., 1997. Genetic identification and status of tilapia regional strains in southern California. Tilapia Aquac. Am. 1, 1–17.

Diaz, R.J., Rosenberg, R., 2008. Spreading dead zones and consequences for marine ecosystems. Science (80-.). 321, 926–929. doi:10.1126/science.1156401

Farrell, a. P., Lee, C.G., Tierney, K., Hodaly, a., Clutterham, S., Healey, M., Hinch, S., Lotto, a., 2003. Field-based measurements of oxygen uptake and swimming performance with adult Pacific salmon using a mobile respirometer swim tunnel. J. Fish Biol. 62, 64–84. doi:10.1046/j.1095-8649.2003.00010.x

Farrell, A.P., 1996. Features Heightening Cardiovascular Performance in Fishes , with Special Reference to Tunas. Comp. Biochem. Physiol. Part A 113, 61–67.

Fitzgibbon, Q.P., Seymour, R.S., Ellis, D., Buchanan, J., 2007. The energetic consequence of specific dynamic action in southern bluefin tuna Thunnus maccoyii. J. Exp. Biol. 210, 290–298. doi:10.1242/jeb.02641

Gleiss, A.C., Wilson, R.P., Shepard, E.L.C., 2011. Making overall dynamic body acceleration work: On the theory of acceleration as a proxy for energy expenditure. Methods Ecol. Evol. 2, 23–33. doi:10.1111/j.2041-210X.2010.00057.x

Halsey, L.G., Shepard, E.L.C., Quintana, F., Gomez Laich, A., Green, J.A., Wilson, R.P., 2009. The relationship between oxygen consumption and body acceleration in a range of species. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 152, 197–202. doi:10.1016/j.cbpa.2008.09.021

Heath, a G., Hughes, G.M., 1973. Cardiovascular and respiratory changes during heat stress in rainbow trout (Salmo gairdneri). J. Exp. Biol. 59, 323.

Johnson, V.R., Russell, B.D., Fabricius, K.E., Brownlee, C., Hall-Spencer, J.M., 2012. Temperate and tropical brown macroalgae thrive, despite decalcification, along natural CO_2 gradients. Glob. Chang. Biol. 18, 2792–2803. doi:10.1111/j.1365-2486.2012.02716.x

Krishna Murthy, V., Dhananjaya Reddy, Y., Manohar Reddy, R., Bhaskar, M., Govindappa, S., 1984. Changes in brain acetylcholinesterase activity and behaviour of freshwater fish in acid polluted environment. Environ. Ecol. 2, 79–82.

Lee, C.G., Devlin, R.H., Farrell, A.P., 2003. Swimming performance, oxygen consumption and excess post-exercise oxygen consumption in adult transgenic and ocean-ranched coho salmon. J. Fish Biol. 62, 753–766. doi:10.1046/j.0022-1112.2003.00057.x

Lehodey, P., Senina, I., Murtugudde, R., 2008. A spatial ecosystem and populations dynamics model (SEAPODYM) – Modeling of tuna and tuna-like populations. Prog. Oceanogr. 78, 304–318. doi:10.1016/j.pocean.2008.06.004

Lucas, M.C., Johnstone, A.D.F., Priede, I.G., 1993. Use of Physiological Telemetry as a Method of Estimating Metabolism of Fish in the Natural Environment. Trans. Am. Fish. Soc. 122, 822–833. doi:10.1577/1548-8659(1993)122<0822:UOPTAA>2.3.CO;2

Mark, F.C., Bock, C., Pörtner, H.O., 2002. Oxygen-limited thermal tolerance in

Antarctic fish investigated by MRI and (31)P-MRS. Am. J. Physiol. Regul. Integr. Comp. Physiol. 283, R1254–R1262. doi:10.1152/ajpregu.00167.2002

Maury, O., 2010. An overview of APECOSM, a spatialized mass balanced "Apex Predators ECOSystem Model" to study physiologically structured tuna population dynamics in their ecosystem. Prog. Oceanogr. 84, 113–117.

Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. a., Bange, H.W., Hansen, H.P., Körtzinger, A., 2012. Future ocean acidification will be amplified by hypoxia in coastal habitats. Mar. Biol. 160, 1875–1888. doi:10.1007/s00227-012-1954-1

Milligan, C.L., Wood, C.M., 1982. Disturbances in Haematology, Fluid Volume Distribution and Circulatory Function Associated with Low Environmental pH in the Rainbow Trout, Salmo Gairdneri. J Exp Biol 99, 397–415.

Perry, S.F., Gilmour, K.M., 2006. Acid-base balance and CO2 excretion in fish: unanswered questions and emerging models. Respir. Physiol. Neurobiol. 154, 199–215. doi:10.1016/j.resp.2006.04.010

Pörtner, H., Bickmeyer, U., Bleich, M., Bock, C., Brownlee, C., Melzner, F., Michaelidis, B., Sartoris, F., Storch, D., 2010. 9: Studies of acid-base status and regulation, in: Riebesell, U., Fabry, V.J., Hansson, L., Gattuso, J.-P. (Eds.), Guide to Best Practices for Ocean Acidification Research and Data Reporting. Publications Office of the European Union, Luxembourg, pp. 137–166.

Pörtner, H.-O., 2002. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 132, 739–61.

Rappaport, A., Sarig, S., Marek, M., 1976. Results of tests of various aeration systems on the oxygen regime of Genosar experimental ponds and growth of fish there in 1975. Bamidgeh 28, 35–49.

Shepard, E.L.C., Wilson, R.P., Halsey, L.G., Quintana, F., Laich, A.G., Gleiss, A.C., Liebsch, N., Myers, A.E., Norman, B., 2008. Derivation of body motion via appropriate smoothing of acceleration data. Aquat. Biol. 4, 235–241. doi:10.3354/ab00104

Van der Linden, a, Verhoye, M., Pörtner, H.O., Bock, C., 2004. The strengths of in vivo magnetic resonance imaging (MRI) to study environmental adaptational physiology in fish. MAGMA 17, 236–48. doi:10.1007/s10334-004-0078-0

Widdows, J., Nasci, C., Fossato, V.U., 1997. Effects of pollution on the scope for growth of mussels (Mytilus galloprovincialis) from the Venice Lagoon, Italy. Mar. Environ. Res. 43, 69–79. doi:10.1016/0141-1136(96)00003-7