Scientific report

"Thermal adaptation in aquatic ectotherms"

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ThermAdapt: Thermal Adaptation in Ectotherms **MADFish – Molecular Adaptation in Fish**

Summary

Aquatic ectotherms are expected to be affected by future climatic changes. However, compared to terrestrial model species and systems for thermal adaptation studies, aquatic ectotherms have only received relatively modest attention and only a few well studied systems are available. Hence, the extent, geographical scale and underlying mechanisms of thermal adaptation of aquatic ectothermic organisms and populations are largely unknown, as is the response to expected future thermal changes. In order to gain a better understanding of these basic research questions, cross disciplinary approaches combining research strategies are desired, but so far, only few inter-disciplinary studies have been conducted in aquatic ectotherms. The main purpose of this workshop was to facilitate interaction between different research disciplines, which have traditionally not communicated very extensively. The workshop was attended by 31 researchers and PhD students spanning research areas of physiology, population genetics, functional genomics, ecology and modelling. Thus, the great diversity of scientific disciplines provided the ideal setting for cross-disciplinary interaction. The workshop programme was divided into thematic sessions and ended with a plenary discussion focusing on future research questions and directions in the field. The many inspiring presentations and lively discussions suggested that this forum is indeed needed and that a cross-disciplinary network should be strongly encouraged. Apart from promoting interaction, such a network should also facilitate future inter-disciplinary research initiatives on thermal adaptation in aquatic ectotherms. During the workshop the participants also identified several research areas which should be prioritized in the future. For instance, there is a clear need for long term studies extending the traditional duration of research projects in ecology and evolution. In addition, the need for common study systems, which could be utilized by different research disciplines, was highlighted. The common consensus after the workshop was a hope that this meeting will be followed by similar events in the future.

Scientific content

The workshop was co-financed with the Nordic network MADFish (Molecular Adaptation in Fish), which addresses many of the same research questions as does researchers involved in the ThermAdapt network. The first day of the workshop began with a MADFish symposium with presentations from members of the network. These presentations addressed various questions related to population structuring and adaptation in fish, including potential effects of climate change on fish populations. The remaining and major part of the workshop was divided into themes covering biogeography and physiological constraints, population genetics/functional genomics, insights from non-aquatic model species, ecological and genetic interactions and bioclimatic envelope modelling. Apart from the latter theme, these topics were represented by presentations by both invited speakers and other workshop participants.

The thermal adaptation workshop opened on Monday evening with two invited presentations, both addressing inter-disciplinary approaches to studies on thermal adaptation in aquatic organisms.

David Conover spoke on counter – and co-gradient variation in life history traits along latitudinal thermal gradients, focusing on extensive work on atherinid fishes. In a literature review, David Conover stressed that counter-gradient variation seems to be common across diverse animal taxa. Applying a combination of extensive common garden experiments and genetic marker analyses in Atlantic silversides, David Conover showed that local thermal adaptation occurs at very fine geographical scales despite seemingly high levels of gene flow between populations. These results suggest that local thermal adaptation may indeed be prevalent despite apparent phenotypic similarities at different locations throughout a species range. Importantly, different life-history traits show different patterns of counter-gradient variation, highlighting the complexity of thermal adaptation in

natural populations. Finally, the potential for extending the framework to a temporal scale in the context of climate change was also addressed.

Later in the evening, Patricia Schulte presented results from studies on killifish along a similar latitudinal thermal gradient in the western Atlantic. Like the Atlantic silverside, killifish represent one of the best studied marine fish species with respect to thermal adaptation. The work on killifish demonstrates the benefits that can be gained by integrating studies on different levels of biological organization, from single cells to organisms and populations. Classical studies have found differences in performance linked to individual gene sequences between populations from northern and southern parts of the distributional range. In a series of studies, Patricia Schulte and colleagues are now investigating the physiological mechanisms underlying adaptive differences in performance at different temperatures. The questions are addressed using an integrative approach including studies of mitochondrial physiology, candidate gene expression, individual performance and population genetic studies targeting both genetic markers supposedly under selection as well as selectively neutral markers. In addition, a natural hybrid zone between northern and southern populations will be targeted in future studies of thermal adaptation in killifish.

The two opening presentations highlighted the usefulness of integrative approaches both across different levels of biological organization and through combining methodologies for studies of thermal adaptation. Although they were both concentrating on fish, the methods and approaches addressed were of a general nature and should thus be transferable to other aquatic organisms as well.

The second day of the workshop was dedicated to presentation on physiological constraints and species biogeography. Hans Pörtner opened the day with an invited presentation about integrating results on various levels of biological organization in order to understand species distributions and potentially project species responses to global warming. Building on studies of temperature effects through oxygen limitation as an important factor affecting distributional patterns in marine species, this presentation also extended discussions to cover effects of changed levels of carbon dioxide. The effects of rising levels of carbon dioxide could interact and exacerbate direct effect of temperature on species distribution patterns in the future. Thus, these results highlight a complicated interplay of effects from different environmental factors and physiological processes under a global warming scenario.

Following this presentation, Wilco Verberk presented background information and project plans for a postdoc project about to be initiated on thermal limits and range distributions in stream invertebrates. Building partly on the theoretical background just outlined by Hans Pörtner, the main purpose of the project is to compare physiological performance and thermal limits between closely related species in order to examine the mechanisms underlying constraints on species distributions.

The next speaker was Takahiro Irie, who addressed the classical temperature-size rule, which predicts larger sizes at lower developmental temperatures. This pattern has been found in many different taxa, but relatively few studies have investigated the relationship in marine organisms. In this presentation, Takahiro Irie presented results from a study of two Japanese populations of marine intertidal cowries. Having a calcareous exoskeleton, growth rate may be restricted at lower temperatures due to lowered calcium carbonate precipitation. Thus, the temperature-size rule may not apply to these species. However, results indeed showed smaller size at metamorphosis for individuals experiencing the highest summer temperatures in nature. Thus, they support the existence of the temperature-size effect in the species, even within a relatively restricted geographical.

The following presentation was by Ignacio Ribera, who presented results from an on-going project investigating the physiological mechanisms underlying range distribution restrictions. Ignacio Ribera is using aquatic beetles as model organisms for studying physiological responses to thermal stress in closely related species with respectively narrow and wide geographical distributions. The presented results indicated substantial differences in protein expression patterns following

thermal stress in the compared species. Thus, results indicated that physiological mechanisms related to thermal tolerance may be involved in determining species distributional patterns.

In the next presentation, Alexandra Cieslak spoke on growth rate during development in cave dwelling beetles. In addition to a number of common proposed life-history adaptations to living in caves, many of these species show relatively large differences with respect to temperature tolerance when compared to other insect species. Thus, there is a very narrow thermal window between optimal temperatures for development and maximum tolerable temperatures. This presentation addressed these issues and discussed future studies to investigate the physiological mechanisms underlying these differences in temperature tolerance.

The final speaker of the day was Tony Wilson, who presented results from fecundity-studies of American and European populations of seahorses and pipefish. Their shallow coastal habitats make these species well suited for studies of thermal adaptation because they are expected to be affected by ambient temperature changes. Tony Wilson presented results from a series of studies combining information from various approaches, such as common garden experiments and mitochondrial marker analyses. Interestingly, while support for Bergmann's rule (increasing body size with latitude) was found for American populations of seahorses along a latitudinal gradient, the pattern was less clear for European populations of pipefish, and Tony Wilson discussed possible explanations for this discrepancy between species.

To summarize, the first day was devoted to presentations and discussions about physiology and life-history in relation to biogeography and species distributional patterns. The presentations reflected the very diverse approaches which are currently applied in the field. It resulted in interesting and general discussions about the mechanisms limiting species distributions and also on potential mechanisms responsible for patchy versus continuous distribution patterns of species. The possibility for extending the framework to an intra-species level was also addressed.

The second full day of the workshop was initiated with presentations on population genetics and functional genomics. Opening the day was Craig Primmer with an invited presentation on rapid evolution in grayling populations in Lesjaskogsvatnet, a Norwegian lake. This particular lake has become a unique study system for adaptive evolution because it was colonized by grayling very recently (approximately 25 generations ago). Earlier studies have found that rapid adaptive evolution is indeed possible in small grayling populations, and Craig Primmer presented results from studies on life-history differences and protein expression addressing the potential mechanisms responsible for local adaptation to different thermal environments. Preliminary analyses have identified several differently expressed proteins between populations inhabiting cold versus warm streams running into the lake. Craig Primmer discussed these findings as well as future work planned on this system. Discussions also addressed methodological issues related to the application of genomic and proteomic approaches in non-model organisms such as grayling.

The next speaker was Einar Eg Nielsen, who presented results from a population genetic study of natural populations of Atlantic cod. The wide distribution of cod exposes populations in different parts of the distributional area to very different environmental conditions, for instance with respect to temperature and salinity at spawning grounds. Results from the applied genome scan approach showed that several genetic markers were likely under selection or linked to genes under selection. Several of these genetic markers were located in genes of known physiological function, for instance heat shock protein genes, suspected to be important in relation to thermal adaptation. Furthermore, genetic variation in several of the genes was associated with temperature conditions at spawning grounds, suggesting this environmental parameter as important for shaping patterns of adaptive population divergence in this species.

Following this presentation, Jakob Hemmer-Hansen presented preliminary results from on-going work on genetic variation in candidate genes for growth and reproduction in Atlantic cod. These genes are suspected to be affected by environmental parameters, such as temperature, which is

varying throughout the distribution of the species. Hence, this represents a gene-targeted approach for genes suspected to be important for local adaptation in natural populations. Preliminary results from a spatial analysis of two populations were presented and the future applications of these markers in relation to studies of adaptation in space and time were discussed.

The next speaker was Kristian Meier, who presented background information and project plans for his PhD on local adaptation in brown trout. This study targets four populations known to be exposed to very different thermal environments. Earlier work using a common garden approach has demonstrated local adaptation to these thermal conditions, and Kristian Meier outlined project plans for a gene expression study to further understand the mechanism underlying these adaptations.

The final speaker within the theme was Erica Leder, who presented the development and results from the initial application of a new microarray for three-spine stickleback. The microarray was designed from publicly available EST sequences and was initially tested in fish from different populations and thermal treatments. Furthermore, the heritability of gene expression was assessed in a second experiment on fish from a breeding set-up. Large differences in the heritability between genes were observed. The results from the initial application of the array were discussed in relation to future project plans.

Collectively, the presentations in the morning session discussed the distribution of genetic variation within species. Several presentations described the presence of variation in thermal tolerance or in candidate genes for thermal adaptation within species, suggesting that species should not be treated as a single unit when attempting to project responses to changing thermal environments.

Following the morning presentations were two presentations on non-aquatic organisms, addressing results obtained with the use of model species. First, Volker Loeschcke spoke on adaptation to stress (including thermal stress) in *Drosophila*. Being among the best studied species for thermal adaptation *Drosophila* species have provided very important information on the mechanisms underlying thermal adaptation. In this presentation, Volker Loeschcke focussed on recent developments in *Drosophila* research. Some of the important recent work has attempted to link results from laboratory experiments to natural conditions. For instance, release experiments have examined survival of released flies in the wild, and laboratory experiments are now applying fluctuating rather than constant temperatures in common garden set-ups.

The next presentation was by Johannes Overgaard, who presented work on cold hardening in *Drosophila* species. While less studied compared to the effects of high temperatures, low temperatures also represent an important temperature stressor in ectotherms. As is the case for studies of increased temperature, studies exploring the effects of lowered temperatures in *Drosophila* have the advantage that it is possible to integrate research across different levels of biological organization. While most research has so far been conducted on the level of individuals, new studies are targeting molecular, cellular and population levels. Johannes Overgaard presented results from physiological studies exploring the cellular mechanism involved in Rapid Cold Hardening as well as studies targeting both ecological and evolutionary time scales. For instance, two studies investigated the Rapid Cold Hardening response on intra – and inter-species levels. Interestingly, no differences were found when comparing different populations of *D. melanogaster* across a latitudinal gradient in Australia, while clear differences were observed between different *Drosophila* species, which were either generalists or tropically restricted.

By presenting important new insights from model species, these two presentations set the stage for discussions on potential future applications in non-model organisms. In particular, new experiences from field and laboratory experiments in Drosophila and other model species could be very useful when setting up experiments in non-model aquatic species.

The third day was split between presentations and a plenary closing discussion on future perspectives. Opening the day were two presentations on ecological and genetic interactions. First, Luc De Meester

gave an invited presentation on climate change effects on *Daphnia magna* populations in meta-communities. Luc De Meester stressed the importance of considering both ecological and evolutionary scales in relation to climate change. Importantly, it has been shown that populations may be able to adapt genetically to increases in water temperatures, but that the evolutionary response depends on the ecological settings. These studies were extended to compare the performance of local northern populations with the performance of introduced southern populations, mimicking a migration scenario under global warming. Here, it was shown that immigrating southern populations could out-compete resident northern populations under global warming, but that this effect was moderated if northern populations were given the opportunity to adapt to increased temperatures before the introduction. These results show that effects of climate change should be predicted through integrating processes occurring on both ecological and evolutionary time scales as well as on local and regional geographical scales.

After this presentation, Philip McGinnity spoke on the interaction between wild and released aquaculture salmon in a climate change context. Several studies have shown that released aquaculture individuals could have detrimental effects on locally adapted natural salmon populations. Philip McGinnity presented results from simulations that indicated that such harmful genetic effects of releases may be exacerbated under global warming. In a particular Irish salmon population, this may result in extinction of the local fish within a 20 year period. Like in the preceding presentation, it was highlighted that local populations may be able to adapt to increased water temperatures. In this case, introduction of non-resident fish is expected to slow this adaptive response, possibly through introgression between the two populations.

The final workshop presenter was Mary S. Wisz, who where invited to talk on bioclimatic envelope modelling. Through two case studies, Mary S. Wisz presented an overview of the potential applications of bioclimatic envelope modelling, stressing current strengths and weaknesses of the approaches and discussed the potential for future integration with population genetic data. The first case study simulated the effects of different climate change scenarios on the distribution of pink footed geese through investigating the effects on both nesting and wintering habitats. The second case study investigated the distribution of harbour porpoises in Denmark in order to predict the potential effects of future placements of offshore constructions. So far, modelling approaches have been working on the species level, but discussions addressed the possibility to move to an intra-specific level. This would be very useful for future applications of the methods for predicting population specific responses to projected temperature changes.

The last day of the workshop closed with a plenary discussion on future research directions. The discussion began with identifying the key questions that future research needs to address. Although knowledge about thermal adaptation in space is accumulating for aquatic ectotherms, we know relatively little about their ability to adapt to future climate changes. Hence, it was highlighted that there is a clear need for research focusing on predicting species and population responses to climate change. Another important question is the degree to which knowledge can be transferred between species, because knowledge is likely to accumulate in relatively few well suited model species and systems.

Most presentation and discussions at the workshop were concerned with the study of thermal adaptation in space, while fewer addressed adaptation in time. However, knowledge of thermal adaptation in space should improve our ability to project response to changes in temperature in time as well. Furthermore, studies in time, for instance through analyses of archived material or real-time monitoring, is also possible and should be encouraged in the future.

Impact and future directions

One of the primary objectives of this workshop was to bring together people from different research disciplines to facilitate cross-disciplinary work in order to improve our understanding of thermal adaptations in space and time in aquatic ectotherms. The above description of the scientific discussions reflects the great diversity of people attending the workshop. This was important because it facilitated valuable interaction between disciplines. There was a general consensus that the meeting had been both constructive and inspiring, and, although no formal projects were initiated during the five days of the workshop, international contacts have certainly been promoted. Moreover, there was a general agreement that similar future venues should be encouraged and that the cross-disciplinary nature of the network should be maintained and extended. Thus, ThermAdapt as well as other networks could function as a lever for future collaborative projects.

Main conclusions

There is a need for future cross-disciplinary research on thermal adaptation in aquatic ectotherms
As evidenced from both presentations and discussions throughout the workshop, questions related to thermal adaptation can only be addressed through collaborations between different research disciplines. This workshop has also shown that there is a large potential for such cross-disciplinary efforts. For instance, physiological constraint models could be extended to also cover interactions between species and perhaps also cover genetic variation within species, and species distribution modelling could be integrated with genetics and dispersal approaches.

There is a need for common model systems which can be studied by several different research disciplines
A common model system would facilitate interaction between disciplines. Some available model systems, such as Lesjaskogsvatnet in Norway, could be well suited for this purpose. However, there is also a clear need to evaluate the extent to which results from one model system can be extrapolated to other systems and species. Results from mesocosm experiments clearly highlight that this should be approached with great care. Thus, while a common model system is important, it will also be necessary to compare results with several other cases in order to evaluate the generalities of the findings.

Long-term funding for networking and projects is needed
Networking should be the lever for future project initiatives. As such, it is important to secure funding for networking activities even though they do not result in new projects *per se*. Moreover, there is a general need for studies with a longer time frame than what is normally possible in ecological and evolutionary research. However, long-term funding for projects may be a major challenge for thermal adaptation studies because funding schemes are typically only covering a few years at the time. Some possibilities for obtaining funding for long-term monitoring could be explored, as could the possibility that valuable long-term data-sets might already exist, for instance at field stations. Future network meetings could address the important funding issues in more detail.

Workshop programme

Sunday 14th June

12.00-18.00	**Arrival**
18.00-20.00	**Dinner**
20.00-22.00	**Social**

Monday 15th June

8.00-9.30	**Breakfast**
9.30-13.00	**MADFish seminar** 9.30-10.00 Einar Arnason: Fisheries-induced evolution and local adaptation 10.00-10.15: Discussion 10.15-10.45: Invited presentation Patricia Schulte: Gene expression differences among closely related populations of Atlantic salmon 10.45-11.00: Discussion 11.00-11.30 Sergey Titov: Genetic differentiation of European grayling (*Thimallus thymallus* L.) from the East part of the area (White, Barents and Caspian sea watersheds). 11.30-12.00 Dmitry Sendek: Whether the origin of vendace (*Coregonus albula*) of the White Sea basin was influenced by introgression of least cisco (*Coregonus sardinella*)? 12.00-12.30 Guðni Magnús Eiríksson: Mitochondrial DNA sequence variation in saithe, *Pollachius virens*, in the North Atlantic 12.30-13.00 Eydfinn Magnussen: Effect of climate change on distribution pattern of fish species around the Faroe Islands.
13.00-14.00	**Lunch**
14.00-17.00	**Arrival**
17.00-18.00	**Start ThermAdapt meeting** 17.00-17.15: Welcome and introduction 17.15-18.00: Invited presentation David Conover: Evolutionary adaptation to climate change across ecological gradients: patterns and processes of countergradient and cogradient variation

18.00-20.00	**Dinner**
20.00-22.00	20.00-20.45: Invited presentation Patricia Schulte: Using intraspecific variation to study thermal adaptation: case studies in killifish and stickleback

Tuesday 16th June

8.00-9.30	**Breakfast**
9.30-12.00	**Biogeography and physiological constraints** 9.30-10.15: Invited presentation Hans Pörtner: Physiological mechanisms linking climate to ecosystem change: effects of ocean warming and acidification 10.15-10.45 Wilco Verberk: Understanding thermal limits of macroinvertebrates by integrating physiology with other aspects of the life history of a species 10.45-11.00:Coffee break 11.00-11.30 Takahiro Irie: Do intertidal ectotherms with a calcareous exoskeleton follow the temperature-size rule? 11.30-12.00 Ignacio Ribera: Identification of physiological adaptations by protein expression profiling under thermal stress
12.00-14.00	**Lunch**
14.00-18.00	**Biogeography and physiological constraints** 14.00-14.30 Alexandra Cieslak: Temperature dependent growth rate during embryonic and larval development in cave-dwelling beetles (Leptodirini, Coleoptera) 14.30-15.00 Tony Wilson: Environmentally-mediated reproductive variation in nearshore pipefishes 15.00-15.15: Coffee break 15.15-18.00 Informal discussions
18.00-20.00	**Dinner**
20.00-22.00	**External lecturers** Kaare Manniche Ebert, Danish Angling Association and Anders Koed, DTU Aqua: Integrating science into management

Wednesday 17th June

8.00-9.30	**Breakfast**
9.30-12.00	**Population genetics/functional genomics** 9.30-10.15: Invited presentation Craig Primmer: Rapid thermal adaptation in European grayling: a synthesis of results so far 10.15-10.45 Einar Eg Nielsen: Genomic signatures of local adaptation in Atlantic cod 10.45-11.00: Coffee break 11.00-11.30 Jakob Hemmer-Hansen: Genetic variation in candidate genes for growth and reproduction in natural populations of Atlantic cod 11.30-12.00 Kristian Meier: Molecular basis of local adaptation in brown trout (*Salmo trutta* L.) 12.00-12.30 Erica Leder: Transcriptional response to thermal stress in three-spine stickleback: Laying the groundwork for thermal adaptation studies
12.30-13.30	**Lunch**
13-30-15.30	**Guided walk in local surroundings**
15.30-18.00	**Insights from non-aquatic model species** 16.00-16.30 Volker Loeschcke: Thermal adaptation and environmental stress: from selection experiments to gene expression studies and field releases 16.30-17.00 Johannes Overgaard: Physiological, ecological and evolutionary aspects of the rapid cold hardening response in insects
18.00 -	**Dinner**

Thursday 18th June

8.00-9.30	**Breakfast**
9.30-12.15	**Ecological and genetic interactions** 9.30-10.15: Invited presentation Luc De Meester: Adaptation to climate change in an evolving metacommunity context 10.15-10.45 Philip McGinnity: Risk of Climate Mediated Extinction Increases when Captive Bred Animals are introduced to the Wild 10.45-11.00: Coffee break

	Bioclimatic Envelope Modelling 11.30-12.15: Invited presentation Mary Suzanne Wisz: Modelling species - environmental relationships for prediction and inference 12.15-12.30: Discussion
12.45-13.45	**Lunch**
13.45-14.30	**Plenary session** *Some working questions* Where do we need additional knowledge? How do we best make use of the networks we have (e.g. through this and other venues)? How can we design studies to be used for predicting future responses of aquatic ectotherms to thermal changes in aquatic environments? Are there scopes for better collaboration and utilization of common genomic resources, e.g. by small consortia conducting 454 seqeuncing of non-model organisms? Actual projects versus networks? Or a combination? How do we finance these projects?
14.30-15.00	**Conclusions/Perspectives from plenary session**

Friday 19th June

8.00-9.30	**Breakfast**
9.30-12.00	**Informal discussions and departure**