Final Report
Leibniz Competition

Aquaculture practice in tropical coastal ecosystems- understanding ecological and socio-economic consequences

Application number: **P78/2014**

Period under review: 2015-2020

Leibniz Institute in charge: Leibniz Institute for Tropical Marine Research ZMT

Project leader: Astrid Gärdes

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1. Achievement of objectives and milestones

ACUTE combined ecological, biogeochemical, health, economic, cultural, and social aspects with a well-defined focus on molecular and disease ecology. This required the establishment of suitable molecular and microbiological tools, ecophysiological assessment of aquaculture practices and societal background information. Our experimental design was based on 1) a combination of field and lab studies as well as experimental model systems, 2) characterization of microenvironment-specific (particles) microbial associations using metagenomic analyses with newly identified marker genes focusing on pathogenicity, 3) quantification of nutrient, organic carbon and energy fluxes in mariculture systems, and 4) evaluation of stakeholder decisions in regard to species and management to develop suitable monitoring systems to prevent pathogen outbreaks protecting human health and livelihoods. Furthermore, we contributed to the basis of sustainable practices and management strategies which will be communicated by the dialogue unit of the ZMT similar to the format we published as Policy brief (Alfiansah and Gärdes (2019) *Towards sustainable shrimp aquaculture.* Policy Brief, 2019/2, Bremen, 5 pp.). ACUTE was subdivided into four work packages (WP 1–4) with several achievements summarized in Figure 1; WP1 elucidated the direct impact of aquaculture sites (fish cage practices) on lateral and vertical transport of particles. The extent of particle transport and flux budgets were determined in the second field phase by in situ optical systems and novel gel traps which can quantify the size and abundance of these fragile micro-habitats. We could show that carbon turnover and microbial respiration rates are important parameters in determining lateral transport and potential hypoxia formation caused by settling of vast amounts of organic matter near the aquaculture site. Respectively, microbial community composition was influenced, which was published in FEMS Microbiology (2019) by Moncada et al. "*Microbial community composition of sediments influenced by intensive mariculture systems".* The quantification of particle concentrations and particle fluxes was performed in different distances to the aquaculture farms to see the environmental stress in comparison to a reference site without direct influence from aquaculture farms. This part was summarized in an article *"Particles in the spotlight: a measure of aquaculture-induced impacts on aquatic ecosystems*" by Hassenrück et al. which will be submitted in May 2021. We confirmed that aggregated organic material can form distinct particles, in which particle-associated microbial communities demonstrate significantly higher metabolic response and functional diversity, and contain higher concentrations of vibrio and fecal indicator bacteria compared to aggregate-free water, which was summarized in three articles 1.) "*Diversity and interactions of gut microbiome of milkfish (Chanos chanos) from open-cage mariculture*", 2.) "*Evaluating impacts of intensive milkfish aquaculture on water quality, organic matter loading, and bacterial communities in Bolinao, Philippines*" by Hassenrück et al., and 3.) Effects of Thermal Stress on the Gut Microbiome of Juvenile Milkfish (*Chanos chanos*). *Microorganisms*. 2021; 9(1):5. Together with WP2 a systematic approach showing that aggregates may be important in the context of disease ecology with respect to the transmission of waterborne diseases from aquatic reservoirs to humans is currently being summarized in an article "*Metagenome assembled genomes of potentially pathogen and antibiotic resistant bacteria from a coastal aquaculture area*" by Hassenrück et al. Here, we distinguished between particle-associated (PA) and free-living (FL) bacteria, because aggregates are known to serve as hotspots for microbial activities and refuges of pathogenic bacteria. Using a metagenomic sequencing approach, we focused on virulence factor genes (type IV pilus, capsular polysaccharide biosynthesis, iron acquisition, extracellular enzyme and toxin), which are well related to pathogen proliferation induced by various aquaculture practices. These analyses were further used as a basis to quantify pathogen proliferation, transfer of antibiotic resistance genes, important for human health and pathogen gene islands using qPCR "*A genus-specific quantitative PCR approach for the detection of pathogenic Vibrio based on the thermolabile hemolysin (tlh) gene"* by Alfiansah et al. will be submitted by May 2021. WP 3 investigated the metabolism of the cultured fish species through both measurements of oxygen consumption

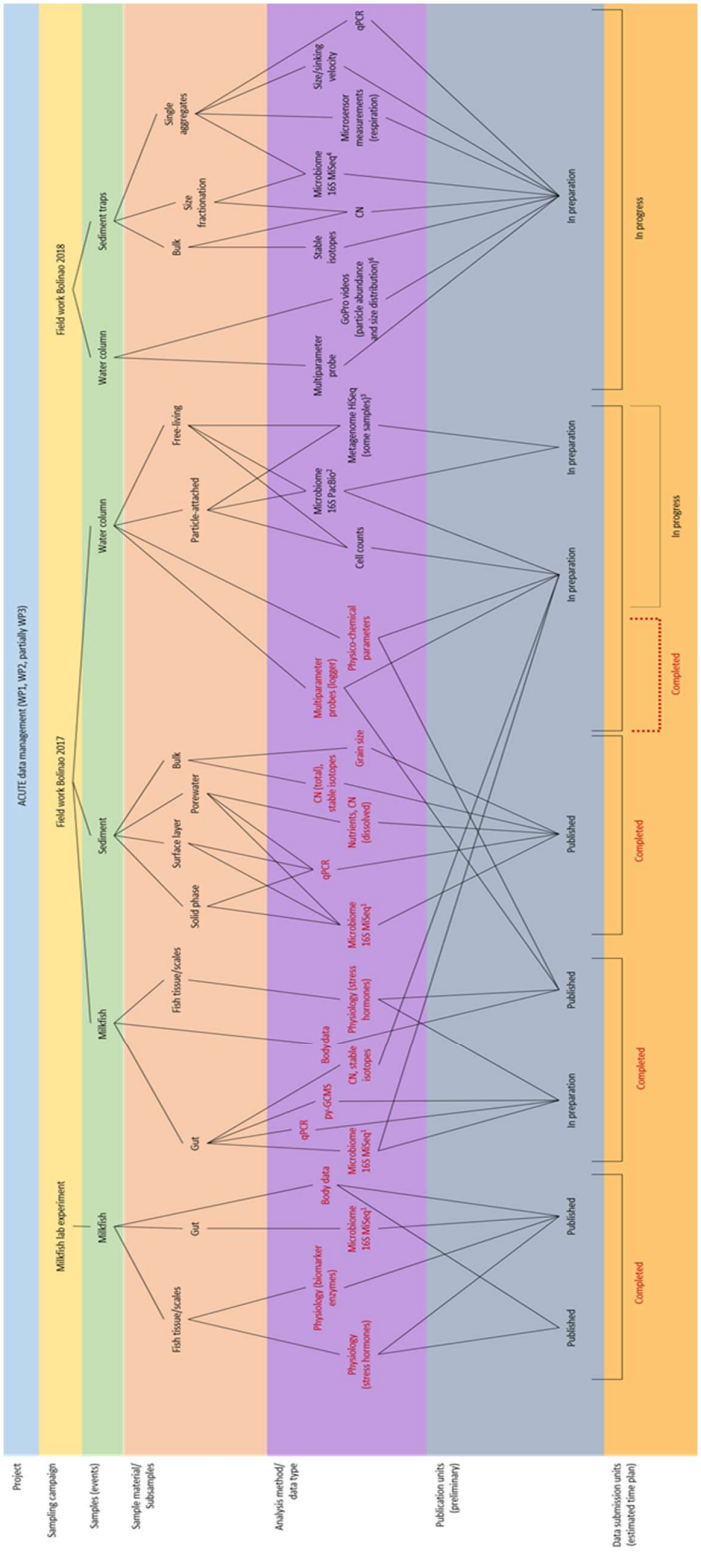

Figure 1: Overview of the data generated within the ACUTE project WP1-3. The data was collected during 2 field campaigns to Bolinao, Philippines, in 2017 and 2018 and during a stress experiment for the milkfish's thermal tolerance at ZMT. The diagram highlights the heterogeneity of the collected sample material and the applied methodology within the ACUTE project to obtain a holistic understanding of aquaculture impacts, and how these data sets were combined in interdisciplinary publication units and associated submissions to public data repositories.

at the organism level with respirometers and at cell level through enzymatic studies on aerobic as well anaerobic pathways. In the course of the experimental design we were able to collaborate with the University of Ghent (J. Aerts) and developed a screening for cortisol levels in fish scales, an indicator for chronic stress published in the Journal Aquaculture (2019) by Hanke et al. "*Thermal stress response of juvenile milkfish (Chanos chanos) quantified by ontogenetic and regenerated scale cortisol".* A comparison of aquaculture practices was undertaken regarding water quality, oxygen and nutrient analyses, using multi-sensor probes and medium to long-term deployment. Analysis of fecal pellets and resulting particle abundance generated the link to WP 1&2. The monitoring of day/night differences and particularly post-feeding periods, but also harvesting periods with increased stress levels for the cultured fish – combined with background measurements of seasonal wind- and current influences was an essential part of this WP, generating another publication in the Journal Aquaculture (2020) "*Chronic stress under commercial aquaculture conditions: Scale cortisol to identify and quantify potential stressors in milkfish (Chanos chanos) mariculture*" by Hanke et al. WP4 aimed at understanding the human dimension, and how the different institutions are governed. With the use of the Social Ecological System Frameworks by Ostrom 2007 a comparative case study during an initial 8-month field stay was carried out analyzing the societal understanding of

industrial aquaculture production. We could show how the two neighboring maricultural districts Bolinao and Anda with similar ecological requirements are differently governed, which lead to less economic loss through major fish kills caused by aquaculture derived pollution in Bolinao. We were not able to understand the complete variety of hard factors, like costs and benefits associated with particular practices or environmental laws, but particularly in a situation where the understanding of the environment, might it be the ecological environment, the market environment or the state environment is characterized by true uncertainty, perceptions, mental models, environmental awareness, network structures or social ties, play as soft factors, a considerable importance how the sector is governed was investigated. The qualitative analysis included participatory observations of involved actors and key-informant interviews of their ecological understanding. Here, the interdisciplinary approach of natural and social science and exchange between WP´s was successful in developing participatory observations using the social ecological systems framework.

2. Activities and obstacles

The ambitious goals of 1) determining the ecological impact from certain aquaculture practices, 2) assessing human health risk, and 3) developing suitable management plans required a consortium of scientists with a) complementary expertise and knowledge, b) well-developed infrastructure, c) existing national and international networks, and d) existing collaborators in aquaculture industries in the tropics. An initial participation of three Leibniz Institutions (ZMT, IGB and ISAS) was intended to offer ideal conditions in terms of methodological knowledge, infrastructure and bioanalytical research facilities. In the progress of the project we decided to improve our chosen methods stated in the application. Instead of developing a microarray (AquaHealthChip) we further developed the technology in using qPCR methods and developed a set of primers specific to pathogens derived from Aquaculture which can cause severe economic damage by emerging diseases. These qPCR methods are sensitive, specific and cheaper and are more suitable in monitoring efforts. We were able to develop these methods at the ZMT and IGB, and therefore the involvement of the ISAS was no longer intended. Initially we proposed our research activities as a comparative study between two tropical countries: i) long-term established aquaculture practices in compliance with legal obligations introduced in 2005 after the extensive fish kill and pathogen outbreak of *Vibrio cholera* in Bolinao, Philippines and ii) long-term aquaculture practices in Lampung Bay, Indonesia with different societal background. The selection of study sites was based on the relevance and compatibility of aquaculture in the respective countries. Although ACUTE started in December 2015, the very difficult and time-consuming ABS Clearance for the Philippines following the Nagoya Protocol took more than 1.5 years of the 3-year project. We were able to import the aquaculture fish *Chanos chanos* from Indonesia and set up aquaria tanks at the ZMT for WP2 and WP3 experiments. This enabled our PhD students to start their reserach without any time delay while waiting for the official letters from the ABS offices. WP4 required a different approach with long-term field stays. Field observations aimed to provide an overview of the study area and to help understand the social, ecological and technical systems from the local stakeholder's point of view. This involved semi-structured interviews to collect primary data from different respondents, namely villagers, fish cage owners, village leaders, related companies, local government officials, province and central (national) government officials, local and national NGOs. Secondary data was collected from annual reports, books, journals, statistical survey reports and other sources published by companies, government and NGOs. Because of extensive required communication with local farmers and other institutions we chose a promising student from Indonesia, Febrina Desrianti, who had shown sure instinct and skills in obtaining the required information for her project work. Unfortunately, her advantage as an Indonesian was rendered moot as our field work was now carried out only in the Philippines. Mrs Desrianti´s exceptional performance in the field, especially as Indonesian Muslim and women in a mostly catholic country, such as the Philippines, was surprising. Nevertheless, other conflicts and challenges related to the requirements of a western University to conduct a dissertation in Germany remain difficult.

Further, health problems had led to several stays of Mrs. Desrianti in her home country which overall delayed her progress in finalizing her dissertation. Mrs. Desrianti is currently in Indonesia while finalizing her dissertation and shall graduate in 2021. Regarding further personal challenges, we recruited a young man for the postdoc position in WP2. He started with great enthusiasms and worked on defining overreaching objectives further linking the interdisciplinary WP´s. Already during our first field travel and workshop at the University of the Philippines (UP) and the Marine Science Institute (MSI) in Bolinao, Philippines (Aquaculture site) it turned out that the very difficult field conditions in tropical countries were not manageable for him. He expressed his fear on longer stays in the tropics and decided to move on in selecting a more suitable postdoc position for him. We hired Dr. Christiane Hassenrück instead, who had some 12-month delay to start her project in ACUTE. Dr. Hassenrück immediately dove into the project and managed her task exceptionally well. Her achievements exceeded our expectations and are conveyed in several publications which shall will be published in due time.

3. Results and successes

The majority of the world´s aquaculture produced fish are from Asian countries, where aquaculture facilities are often characterized by the heavy use of chemicals, antibiotics, overfeeding, and little environmental awareness and control. Important processes regarding waterborne diseases, which cause extensive ecological and economic damage and harm human health, were of special interest in ACUTE. We have focused our studies on open-cage fish aquaculture of *Chanos chanos*, investigating the fish's stress response, composition of gut microbiota including the identification of important pathogens and potential health risks associated with the consumption of cultured fish due to the use of important human antibiotics, disease emergence and environmental impacts caused by aquaculture facilities. This required a need for coordinated research in a holistic social and natural science approach to identify critical control points. Additional to the publication outcomes of ACUTE described in section 1. two MSc thesis and two PhD thesis were completed in 2018 and 2019 respectively. A third PhD Thesis will be finalized in 2021. Primary results were presented in numerous conferences but was also an integral part in industry events, such as "Science meets Business in 2019", the Science Sips Event on "Taste of the Future of the Sea" in the Science Year 2016*17 – Seas and Oceans, and several contributions as "Expert Opinion" by the PI to the Science Year 2017. Our outreach and contributions resulted in several funding possibilities granted to the PI Astrid Gärdes with an overall budget of over 440.000 Euro as PI an additional 300.000 Euro as Co-PI. The ZMT research group around the PI Astrid Gärdes has been involved in the Leibniz Research Alliance (LRA) "INFECTIONS'21 – Transmission Control of Infections in the 21st Century" since January 2016 as a consequence of the funded ACUTE project. Fourteen Leibniz institutes and three external partners are involved in this interdisciplinary alliance that carries out research on the control, prevention and therapy of infectious diseases. The LRA includes not only infectious disease research dealing with the causative agents of diseases to find new therapies, but also addresses issues like environmental conditions which promote spread and transmission of the infection, which have long been part of this endeavor. Together, the LRA partners discover new issues, create and link additional networks and promote the involvement of their own international collaborators into the projects. The alliance's outreach is consequently becoming much wider. For example, no comprehensive inventory and control of antibiotics and other antimicrobials exists. Therefore, it is unclear which vectors of antibiotic-resistant bacteria exist, which complicates screening for specific bacteria also in aquaculture environments and aquaculture products. The outcomes of ACUTE highlight the importance of antibiotic resistance especially in the genera *Vibrio* and *Acinetobacter*, and contributed as such to the successful application of the LRA Infections ´21 for second funding phase focusing on antimicrobial resistance (AMR) in pathogens and their transmission. ACUTE was also part of the Research Alliance Sustainable Food Production and Healthy Nutrition. Project partners have been successful in acquiring the project: Food for the Future funded by the Leibniz Competition. Last, as a success of the Leibniz funding line *Women Professors*, the PI Astrid Gärdes received a joint professor position at the Applied

University in Bremerhaven and the Alfred Wegener Institute for Polar and Marine Science AWI in 2020.

4. Equal opportunities

Ensuring equal opportunity and a non-discriminatory working atmosphere for women and men was a central part of ACUTE. We promoted female and male scientist equally, resulting in 90% female PhD and MSc students, female PI on both sides, in Germany and the country of our study site, the Philippines, and the even distribution in gender among senior scientists.

5. Quality assurance

All data sets have been or will be, respectively (Fig. 1), archived on the appropriate recognized data repository (Pangaea, ENA, MetaboLights) using the data brokerage service GFBio, if applicable. All data submissions followed the FAIR guiding principles for data management and have been conducted in compliance with established metadata standards (MIxS, ISAtab). Furthermore, the computational data analysis scripts have been archived alongside the data in commitment to open science and to maximize the reproducibility of the published studies.

6. Additional in-kind resources

The host Institute ZMT provided 40% in personal cost, in total 392.912,48 Euro from a total fund of 989.280 Euro.

7. Structures and collaboration

The Marine Science Institute (MSI) of the University of the Philippines maintains the Bolinao Marine Laboratory in Bolinao, Philippines. Thus, collaboration with scientists of the Marine Science Institute, specifically Cecilia Conaco who was introduced at the beginning of the project replaced Wolfgang Reichert who had just retired, and was an integral component of the project. Secondly, partners from the Aquaculture Centre in Ghent as part of the University of Ghent, Johan Aerts, contributed significantly to WP3. Our initial research partner Albert Sickmann from ISAS and Carsten Schulz from GMA were no longer essential in completing ACUTE as experiments could be carried out at ZMT facilities. Instead, for the quantification of antibiotics residues Stefan Effkemann and Edda Bartelt of the Institute for Fish and Fishery Products, Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), Cuxhaven, became involved. As conveyed in the publication record, joint publications among ACUTE partners were established for all publications, with emphasized contribution of the Leibniz Institute IGB by Hans-Peter Grossart.

8. Outlook

The entire project is embedded into a stakeholder dialogue, in which the acquired natural and social science knowledge will provide an important input not only for the particular case of aquaculture, but also beyond. In the face of regional and global AMR challenges and part of the new EU One Health Action Plan against Antimicrobial Resistance our method development for the detection of pathogens in environmental samples through high-throughput qPCR techniques will contribute to efficient monitoring of potential health risks. State-of-the-art metagenomic sequencing technology and bioinformatic analysis pipelines can contribute in developing and expanding existing virulence and AMR databases by information on sequence variants of existing resistance markers. Multivariate analysis and different modelling approaches will enable predictions and risk assessments based on the multiple datasets generated in the project. Overall ACUTE tackled pressing topics in EU One Health Action Plan.