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Thinking Through Experiment: Enacting RNAi

In the preceding, I told a story about the emergence and organization of a novel experimental system for investigating questions in lice biology, and how the social and technical conditions for production of such knowledge coalesced in the Sea Lice Research Centre. Taking a cognitive-historical approach to this problem-solving complex, I situated a range of epistemic activities in their context. This analysis was couched in concepts drawn from science studies on the cultural diversity of experimental knowledge, occasionally invoking the language of distributed cognition and related "environmental perspectives" on the scientific process (Nersessian, 2009).

A focus has been on how scientific instruments and concepts become meaningful when inserted into a historical context of experimentation, capable of differential reproduction through repetition, variation, and iteration (Rheinberger, 1997, 2010). But a detailed account of how novel scientific meanings arise and propagate through instances of explorative inquiry, from which new bits of knowledge emerge, requires a different level of analysis than the one employed so far. In the next three chapters, I therefore shift focus and present a series of interactional analyzes based around cognitive ethnography that animate concrete episodes

M. Solberg, *A Cognitive Ethnography of Knowledge and Material Culture*, Culture, Mind, and Society, https://doi.org/10.1007/978-3-030-72511-2_5

observed in the lab. The goal is to give an ethnographic account of what information goes where, when, and in what form, during instances of bench work at the Centre. I do so, by underscoring how scientists together construct, develop, and maintain ecological assemblies within the cultural-cognitive ecosystem of the SLRC, spanning from the performance of RNAi experiments (this chapter) to measurements of gene expression (Chapter 6), and collaborative microanatomy of lice tissues (Chapter 7).

Springing from the framework of distributed cognition, cognitive ethnography's guiding heuristic is to ask about a given activity such as a lab experiment, "what information goes where, when, and in what form"? The basic procedure consists of first identifying a relevant cognitive task, and then using ethnographic insights to find out which elements play a causal role in completing the task by subjecting these components to a functional, interactional analysis. As a practice-based method, it samples the unit of analysis from naturalistic events. A primary concern is how agents use and coordinate conceptual resources with the material resources of their external surroundings to think, act, and construe meanings. These phenomena of interest span from social interactions manifested through language, via the use of gesture, to the manipulation of material artifacts, and so on. A central premise is that the meanings of actions are grounded in specific contexts of activity, which cannot be surmised from studies on cognitive processes in "captivity" (Hollan et al., 2000: 179).

This approach draws on the ethnographer's toolkit for attending to everyday interactions in peoples' lifeworld to better understand the nature of cognition. It examines how events unfold in communities of practice by extending the unit of analysis from individual minds to the interaction and propagation of representational states through various representational media in larger social systems, environments, and across time. By conceptualizing the laboratory as a cognitive ecology, the ethnographer of scientific knowledge can literally step inside and behold how experimental systems become "elaborate filters set up in the space of phenomena," to invoke a salient metaphor (Galison, 1987: 13).

Rooted in traditional ethnography, cognitive ethnography expands its reach and scope by taking seriously the interactive nature of meaning

and knowledge construction. Using digital video, it becomes possible to capture minutiae of real-time activities and to analyze dynamic interactions, thus complimenting classical participant observation with more elusive data points. These may, in turn, be augmented by historical materials, studies of artifacts, written media, interviews, and other items from the ethnographic toolkit. Together, these resources can redress human shortcomings in intuition and memory that inevitably follow when people self-report on the dynamics and structure of their own multimodal, semiotic activities. Often, these go unnoticed and they are too fleeting to be captured by ethnographic observation without technical augmentation (Alač, 2011).

This workflow, which Hutchins calls a "cognito-scope" for observing cognition in the wild (2014), starts out with regular participant observation of conduct in a community to identify patterns of important activity and gain insights of relevance for later analysis. After samples of naturally occurring events have been observed and recorded on video, the structure of events is then indexed, and scanned for salient segments of interaction that cast light on a given situation. On this basis, a selection of specific micro events is transcribed using multimodal transcription schemes, depending on the phenomenon of interest. It is also possible to align renderings of still-images from relevant interactions with transcripts to support further analysis (see Alač, 2011), as I occasionally do in the following. As such, cognitive ethnography attaches "descriptive comments" to ethnographic representations; "directions for use" that invites the reader to evaluate both interpretations and theoretical inferences (Sperber, 1985).

Disciplined attention to fine-grained accounts of talk, uses of artifacts and other forms of bodily interaction at the microlevel of social contexts, originates from conversation analysis of natural discourse, studies of speech-in-interaction, and ethnomethodological approaches (Goodwin, 2000; Streeck et al., 2011). Using video analysis in science studies offers an alternative route for grasping how mundane resources participate in local experimental accomplishments, and avoids the risk of "theoricism" (Sormani et al., 2016: 128). That said, the question of who is "doing what, why and how" in the performance of laboratory action only becomes meaningful when contextualized through immersive participant

observation over time (Chang, 2011). Like other methods in the human sciences, it is also selective in scope. It is not possible, as Goodwin underscores, "to work in some abstract world where the constitution of knowledge through a politics of representation has been magically overcome" (1994: 607).

Ordinary ways of thinking about knowledge as a property of individuals tend to break down when we examine aspects of contemporary science, a technology-driven and distributed enterprise involving many minds (Giere, 2002: 644). In their laboratories, scientists collectively mediate interactions with physical nature through a wide range of semiotic modalities and external representations that order access to the world (Coopmans et al., 2014). According to distributed cognition, our cognitive faculties fundamentally depend on the ability to engineer such external props to scaffold thinking. Echoing Hutchins, we can say that scientists build their cognitive powers in part by creating problem-solving environments where they exercise these powers (Nersessian, 2012: 223). Or in this case of molecular parasitology: by engaging an experimental system comprising domesticated organisms, instruments, other researchers, and a suite of epistemic activities. In these environments, arrangements of instrumentation and concepts are "laminated" through layers of semiotic action that eventually make epistemic things meaningful (Goodwin, 2013).

A primary function of this cognitive ecology is transformation of representational structures. Experimental science does not just encompass mental representations found solely inside the head as disembodied theory bounded by the epidermis, but fundamentally involves rearranging things in the laboratory to reveal informative patterns, by building external models through a suite of discursive practices that make unknown phenomena intelligible. When studying this cognitive niche, where scientists inherit resources from their predecessors that help enact new material transformations on the world to better understand and explain it, we must attend to representation and re-representation as both product (noun) and practice (verb).

In this chapter, I examine how RNA interference experiments are arranged in situ to produce and transform representations that mediate

the phenomenal objects of gene expression in salmon lice. By phenomenal objects, I mean the features and outcomes of experiments, that are the professional concerns of these biologists. In this, I build on Goodwin's insight: "central to the social and cognitive organization of a profession is its ability to shape events in the domain of its scrutiny into the phenomenal objects around which the discourse of the profession is organized" (1994: 626). I trace this shaping of events through critical steps in what my interlocutors colloquially referred to as their "pipeline"; the assembly line of material, conceptual, and virtual interventions on lice that transform RNAi experiments into useful data and insight. Despite the ubiquity of external representations in experimental systems, their status is often taken for granted by insiders in a scientific community. Inscriptions like numbers, letters, notational systems, diagrams, drawings, images, and other visualizations, appear to litter the work environment, simply as historically accumulated products of human activity. But getting them to work in a coherent, mutually supportive fashion, requires practical engagement across multiple semiotic fields. As such, they are far from trivial, but core elements in the architecture of scientific perception.

But what turns material representations, like squiggles on a piece of paper, a label, or a digital spreadsheet into productive, meaningful representations within a given experimental system? The answer to this question is "enactment," as material patterns achieve representational functions through engagement in a culturally shaped perceptual process. The phenomenological worlds of skilled scientists are not made up of isolated objects, but orchestrated in systems of "enacted understanding" (Hutchins, 2010: 429–430). Competent professionals, experimental scientists included, apply three key discursive practices to enact meanings (Goodwin, 1994). First, they use coding schemes to transform salient phenomenon into knowledge objects that animate professional discourse. Secondly, they mark out specific things for attention, often under complex perceptual circumstances, by highlighting them. And third, professionals articulate material representations in the domain of scrutiny to co-produce phenomenal objects of interest. Through such multimodal enactments, experimental systems jointly engage perception, action, and imagination (Hutchins, 2010).

A view of scientific representations as enacted, resonates with Hacking's taxonomy of elements contributing to the "self-vindication" of the laboratory sciences (1992). His materialist thesis state that the laboratory sciences create mutually supportive structures consisting of ideas (intellectual content), things (including targets, sources of modification, detectors, tools, and data-generators), and the manipulation of marks (data assessments, reductions, analysis, and interpretation). But although Hacking's inventory draws attention to what makes experimental practices cohere, I mentioned in Chapter 1 that this vocabulary lacks the grammar to go with it (Chang, 2011: 206). What is further needed in studies of experimentation in the wild, is a thesis taking seriously the relationships between "thoughts, acts and manufactures" (Hacking, 1992: 30). Distributed cognition is precisely a thesis about such relationships, and cognitive ethnography offers tools to investigate how they are socially arranged in practice.

Here, I flesh out this relationship empirically, through an ethnographic study of how representations are enacted in an activity system that encompasses the initiation and termination of RNA interference trials. Examinations of events, sampled from the preparation and execution of these experimental events, explore how RNAi trials establishes meaningful relationships between bioinformatic resources for digital handling of genetic sequences, lice samples, instrumentation, engineered nucleotides, sorting systems, written representations, and other scientific visuals like annotations, Excel spreadsheets, and digital photographs. By asking, what information goes where, when and in what form within these paradigmatic interactions at the Center, I show how novel scientific meaning emerges from laboratory organization. To do so, I must attend to the experimental system's representational states, and how the configuration of information-bearing structure changes over time. Ethnographic description of events ties together relations between disparate elements that only come together during certain moments of practice, and which are hard to articulate by members of the community when prompted outside the situated context of action.

In the labscape, representational structures in one medium are systematically transformed and re-represented into structures in a different medium. RNAi trials align the mental models of scientists with biological

samples, analog and digital inscriptions, verbal utterances, gene expression measurements, information from histological tissue sections and biochemical visualization methods. These alignments create interlocking models that support insights about relevant target structures. Theories of distributed, situated, and extended cognition suggest that such interlocking models indiscriminately combine internal and external resources, artifacts, and practical action. Applying the "cognito-scope," we see how the laboratory organization of the SLRC effectively sets up an ecology for exploring epistemic things in an interdependent space, satisfying the constraints of different resources in the system. Invoking the ecological metaphor here, allows us to think about interworking elements, and how they relate to epistemic outcomes. These scientific practices are fundamentally social, as they are coordinated and constrained by the practices of other humans, animals, and artifacts within a wider cognitive ecology. In this context, to "coordinate" simply means to "set oneself up in such a way that constraints on one's behavior are given by some other system" (Hutchins, 1995: 200). Rather than seeing the propagation of representations in the experimental environment exclusively in the narrow terms of "information transfer" between individual cognizers, I find it productive to view these accomplishments as alignments of resources in the experimental system. Attending to RNAi trials here, I use the next two chapters to examine how other resources for representing and intervening on lice biology get coordinated downstream in the experimental pipeline.

Orchestrating Molecular Manipulations: The Checklist

Although the lice genome was already sequenced, scientists only had indirect information about the biological role of individual genetic sequences in the living animal. As explained in Chapter 4, the Centre therefore conducted two types of RNAi experiments aimed to silence specific genes, so that more could be learned about their function. Between 2012 and 2018, the Centre executed 396 such experiments on lice at the nauplius stage (aimed at 234 different gene targets), and 380

experiments on the pre-adult stage (aimed at 330 different gene targets), not counting controls and replicated experiments (SLRC, 2018).

To probe gene expression in early planktonic stages of the life cycle, the group used a soaking method. Larvae at the molting stage were immersed in a sea water bath, to which synthesized double-stranded RNA fragments had been added. These fragments were then absorbed across the larvae body and set in motion the RNAi machinery to silence the targeted genes. As these experiments were relatively simple in terms of the necessary resources, they could be executed by individual scientists without much planning and coordination with other lab members. The more conspicuous screening events were those based on direct injections of pre-adult louse specimens. In these experiments, double-stranded RNA molecules were injected into large batches of salmon lice harvested from live fish, that were then reinfected to the salmon hosts, and placed in the single-tank system to maintain tight control over experimental outcomes. These events were laborious and required coordination between several researchers since the accompanying tasks were time sensitive. Here, practical constraints inherent to the experimental context necessitated a division of labor spread across two complementary task domains: while one group of researchers primarily handled nucleic acids and salmon lice, another group cared for the host fish.

When taking the distributed perspective on experimental science there is a need to distinguish between two categories of cognitive labor involved in the execution of tasks that are spread across a community of practice. First, there is the cognition that is the task itself. Secondly, there is the cognition that governs the coordination of participants, elements, and actions involved in executing the task. In the everyday flow of situated research activity, these interact as to create emergent phenomena whereby "the group performing the cognitive task may have cognitive properties that differ from the cognitive properties of any one individual" (Hutchins, 1995: 176). Note that while my focus in the following is on coordination of elements involved in large-scale RNAi trials on pre-adult specimens, this does not imply that there is no distributed cognition occurring in the smaller RNAi experiments where individual scientists apply the bath method at the nauplii stage. However, given my

ethnographic interest in epistemic activities on the level of the experimental machinery, I find conduct in these joint trials to be a particularly relevant unit of analysis for teasing apart how complex RNAi experiments make knowledge. I return to the cultural practices of cognition, and the ecological assemblies that enable situated individuals to accomplish computational spread to solve complex bench work, when we move further down the experimental pipeline.

How does an experiment produce a set of tractable representations that can lead to novel insight about biological phenomena? Getting research done within the constraints of SLRC's experimental system required copious amounts of work. Since the pipeline for large-scale RNAi had limited capacity, a senior scientist at the Centre was responsible for scheduling and keeping track of all past, ongoing, and future RNAi-screening experiments, and their main outcomes. This job involved gathering information from all the other researchers and students about the lice genes that people worked on, individually and jointly. To ensure uniform and coherent execution of these experiments, a public account spelled out how people, nucleic acids, artifacts, salmon lice, and salmon should be coordinated before, during, and after RNA trials. This document, which took the form of a normative and prescriptive checklist (hereafter referred to simply as "The Checklist"), was a "regulatory representation" that established RNAi trials as a particular cultural institution (Heintz, 2007). Building on Dan Sperber's epidemiology of representations, Heintz proposes that communal institutions of this kind get their identity from causal chains that distribute representations so that they cause and structure reoccurring events. In this perspective, an institution like The Checklist is defined as "the distribution of a set of representations which is governed by representations belonging to the set itself" (Sperber, 1996: 76). Institutions play an important role in distributed cognitive systems, as these both provide mechanisms that ensure the social reproduction of the system over time by regulating its function, and a distribution of representations that fulfil this function.

The Checklist circulated within the Centre as a public document embodying a cultural script that regulated how other representations ought to be processed within the socially legitimate performance of

an RNAi trial. The script spelled out by the document had normative force, providing authoritative instructions for the sequential nature of events and actions, how information should be codified in inscriptions (what information should be written down, which things should be labelled, and how), how artifacts, animals, people, and inscriptions should be coordinated and propagated, and not least: procedural descriptions for a range of different pragmatic and epistemic actions. As a regulatory representation, The Checklist also functioned as a "coordination device," controlling how representations within the activity system should propagate, by exerting constraints on possible moves in the sequence of action (Hazlehurst et al., 2007: 543). It achieved these regulatory effects by laying out an arrangement of resources for RNAi, specifying courses of action in five temporal orders: "preparations," "day of injection/initiation," "monitoring of experiment," "termination," and "hatching."

Choosing a Fragment

Let us start with the early preparations of nucleotide fragments. Before an RNAi trial, all participants must figure out which gene they want to silence. As The Checklist instructs: "find out what gene you want to knock down." How did my interlocutors accomplish this?

Several routes were available for homing in on relevant lice genes for experimentation. And like with other scientific accomplishments these were constrained by the Centre's division of labor. In science, division of cognitive labor is institutionalized on multiple levels, with macro-level divisions between disciplines (biology, physics, chemistry, and so on), within sub-disciplines (molecular biology, ecology, zoology), laboratories, and research groups, and within specific projects (Muldoon, 2013). At the SLRC, fine-grained labor divisions were necessary due to the biological complexity of lice, and the great diversity of candidate genes that could potentially be subjected to screening experiments using RNAi. Work Package 4 crystallized around three main topics of interest: host–parasite interactions, basic copepod biology, reproduction, as well as the endo and exocrine systems of salmon lice. These biological

domains were considered the most epistemically rewarding, with a high chance of resulting in therapeutic breakthroughs. Work in each topic was also coordinated on a more fine-grained level, including:

- *Genes related to molting, and general parasite growth*. One doctoral student worked on a class of genes related to a protein known as fibronectin (which we encounter in the next chapter). Another examined the function of genes predicted to be involved in chitin degradation. Chitin is a necessary component of exoskeletons, and enzymes called chitinases are required to degrade chitin when the parasite molts, making them popular targets for pesticides in both terrestrial and marine environments.
- *Endocrine regulation of ontogenesis*. One Ph.D. candidate extensively characterized the ecdysone receptor (EcR), using RNAi and other methods. This receptor was believed to be involved in biological mechanisms such as ligands, a type of hormone substance that binds to other molecules, forming larger complexes. If these receptors could be shown to act on key developmental transitions like molting, they would be interesting therapeutic targets. Part of this work involved an attempt to quantify steroid hormone levels through a novel assay developed in collaboration with staff from Work Package 1, based in Oslo. Another postdoctoral project worked on genes involved in the ferritin pathway, an intracellular iron transporter protein that was assumed to be central in iron regulatory processes. In this work, the postdoc screened fourteen different candidates involved with heme, a prosthetic group that binds to proteins like hemoglobin, using RNAi, as well as genes related to iron metabolism.
- *Germ-cell differentiation and maturation*. One Ph.D. candidate worked on the Nanos gene family, which are crucial for germ-cell development as they bind to mRNA molecules and block translation of key proteins necessary for growth and differentiation. While this project was eventually relinquished, another candidate examined genes involved in oocyte-formation, yolk deposition (vitellogenesis) and lipophorin regulation, transport, and uptake. These processes were central for bringing proteins and lipids for the growing embryo into the developing eggs (oocytes).

- *Chemosensory genes involved in host recognition*. Genome sequences revealed that the parasite lacked typical chemosensory receptors found in other species. A postdoctoral project examined the function of receptors suspected to process chemical communications with the environment, such as detecting host fish, and genes involved in chemoreception, reproduction, and lice behavior.
- *The molecular biology of exocrine gland structure*. A fourth postdoctoral candidate spent considerable time mapping these anatomical structures, as well as characterizing viruses shown to be present specifically in lice glands. We return to this work in Chapter 7.
- *Immune modulation*. A team of junior and senior researchers at the Centre characterized Prostaglandin E2 synthase (PGE2), an immune modulator suspected of inhibiting inflammatory responses in many parasite hosts. Despite much effort, the group failed to elicit any changes in phenotypical or reproductive output from lice after conducting RNAi trials on this gene.

The cognitive and practical divisions of labor exemplified by these efforts can be viewed as an epistemic and economizing strategy of "risk-hedging" through diversity in the context of scientific discovery (Muldoon, 2013: 123). As the Centre promoted a diverse portfolio of projects on a variety of critical genes and biological processes, priorities were set by individual researchers, who were specialists on their topics of interest in close dialogue with senior leaders, thereby combining a centralized, well-organized search based on the Centre's strategy with more local judgments concerning best practices and methods. Generating and maintaining this diverse portfolio was epistemically rational, despite the burdens associated with successfully coordinating it, since it was impossible to predict accurately which of the experimental targets would materialize as tangible breakthroughs.

Thinking Through Trees: Phylogenetics as Epistemic Enhancers

Two additional examples illustrate in detail the general schema for the selection and exploration of RNAi targets. My first example concerns a study that began with an evolutionary insight. It turned out that a type of receptor, here dubbed "R," is crucial for a cellular process related to gene regulation in all metazoan animals. While many studies had investigated this biological mechanism in other species, there was little knowledge about its role in salmon lice biology. With knowledge about genetic sequences involved in the R pathway from other genomes, a search for matching sequences was then performed in the louse. This yielded several hits for similar genes, including variants involved in general growth processes, as well as reproduction. Complete transcripts of these genes were then identified using a method known as RACE ("Rapid Amplification of cDNA Ends"). Further sequencing found differences in the domain's genetic structure, and quantitative PCR identified locations where these genes were highly expressed in the lice body. It turned out that the level of transcription and its location varied significantly through the life cycle. While some transcripts were identified in the gut, others were found in the reproductive system. The genes also appeared to express differently in males and females. An RNAi experiment probed the effects of silencing the expression of R, which yielded new insights about its biological function. Of interest, was the fact that RNA interference appeared to eliminate viable offspring in adult females. Further studies showed that many other reproductive genes were also disrupted by silencing R. Indeed, the use of "wide instruments" like the microarray, later showed a large effect on many diverse genes. Besides offering valuable insights about the function and evolution of this biological mechanism, the investigation concluded that R and downstream genes could be potential targets for therapeutic interventions, making them attractive for further study.

A second example concerns P, a protein previously described as an active modulator of the host immune response in ticks. It was suspected that P could also be active in the louse. In fact, a study of P had been conducted earlier by a competing research group abroad, but results were

inconclusive. Scientists from the SLRC had some novel ideas about how to improve on this research. First, P in lice was characterized at the molecular level, using a similar approach as the one used for R. Complete transcripts of a central gene involved in producing P were obtained via RACE, and computationally compared to a similar protein that was identified in 39 other species. An evolutionary (phylogenetic) model of these relationships was also compiled, along with an expression profile of genes believed central for producing P. When the patterns of gene expression in developing lice tissues were visualized, it turned out that the gene was expressed both in muscle cells and in the reproductive system of adult female lice. The group then decided to silence the production of P in specimens at the nauplii stage using the bath treatment, and adult lice using the injection method. These experiments showed no significant effects on nauplii nor adults, and the group concluded that observed expression patterns did not conform with the observations of previous studies. The disruption of P appeared to not affect any essential functions under these circumstances.

These two examples condense important features about how candidate genes were identified and subjected to experimental investigation through the pipeline. As an invertebrate with an exoskeleton, a segmented body, and jointed appendages, *Lepeoptheirus salmonis* is grouped among other crustaceans, arachnids, insects, and myriapods in the phylum of arthropods. From a parasitological perspective, genes in salmon lice are particularly salient objects of inquiry when they are involved in biological processes, whose associated mechanisms could be targeted by either vaccines or other therapeutics used against parasitic arthropods. Research on insect pests in other domains, where the sheer amounts of invested workhours dwarfed those of marine aquaculture, thus offered a scaffold of knowledge for parasitological work on the louse. It was not uncommon that therapeutics originally developed for terrestrial agriculture and husbandry could be successfully transferred to marine fish farming. The most appealing targets for vaccines and other pharmaceuticals would be those working on a narrow class of organisms, since indiscriminate side-effects could potentially affect the marine ecosystem negatively. This had long been problematic for some drug classes, such as chitin-synthesis inhibitors, which were suspected

to adversely affect a broad spectrum of crustaceans around salmon farms, including commercially important species like shrimp, crabs, and lobsters. This challenge made a vaccine against the louse especially salient, since vaccines work by stimulating the immune system to protect individual fish and would thus reduce environmental impacts compared to other therapeutics, like drugs added to fish feed or pesticides used in bath treatments in farms.

By searching the genome for genetic sequences involved in critical adaptations for the parasitic lifestyle, like those playing key roles in reproduction or regulating host interaction, it was possible to narrow the search space considerably from the roughly 13,000 genes that were identified in the louse. One way that scientists at the Centre could narrow this space of possibility and make qualified decisions about suitable targets for RNAi screening experiments, was to engage in evolutionary reasoning about the descent of sequence, as exemplified in the two examples above.

Biologists and laypeople alike, classify and order salient discontinuities between animals and plants hierarchically in taxonomies, in groups within groups (Ellen, 2004). Together with causal cognition and inference (the ability to go beyond available information), classifications are fundamental for learning about the world. Anthropologists of knowledge have long concerned themselves with the universality and structure of taxonomic reasoning and its degree of cultural infusion (Ellen, 2006). Atran, for instance, proposed that folk taxonomies are based on stable cognitive schemas, a "universal domain of cognition that produce special forms of worldly knowledge" (1990: 253, but see Ellen, 2004: 422–425 for a critical discussion). This continuum hypothesis of knowledge suggests that both laypeople and professional biologists draw on commonsense intuitions, like the folk notion of "species," when thinking about living kinds. Within the subfield of systematics, biologists have argued vigorously about how to order biological diversity in taxonomies and nomenclature (the appropriate rules and criteria for naming entities at different taxa), and how they should be ranked and ordered in classifications. While several traditions in systematics competed throughout the twentieth century, the approach known as "cladistics," or "phylogenetic systematics" became dominant as the majority of biologists agreed that the most effective classification was one reflecting the history of

Darwinian evolution (see Hull, 2010). In this scheme, organisms are assorted monophyletically into groups nested within groups descending from a single ancestor, a perspective that modern bioinformatics extends to the molecular level. Before DNA sequencing became widespread, the topic of phylogeny was mainly the providence of systematists. Today, however, it is widely used across all subfields dealing with sequence data. Phylogenies do not only describe evolutionary relations at the species level, but also helps understand the relationship between genes and their products.

Since Darwin, genealogical relations between organisms have commonly been represented as a branching tree, a motif with a long social history in various cosmologies, as revealed by a rich collection of cultural productions about living kinds. For instance, in his ethnography of marine microbiologists, Stefan Helmreich describes how the science of gene transfer in extremophile organisms dissolves assumptions about the evolutionary roots on the tree of life (2009, Chapter 2). Tree-like representations based on models of molecular evolution can diverge quite radically from folk intuitions about species relations. When reasoning reflectively about biological ancestry, professional biologists do not consider the species-level to be a container of essences (as laypeople tend to), but rather view this taxon as a construct for pragmatically grouping certain things together. For those working with phylogenies the main preoccupations are "clades," monophyletic groups of organisms and sequences encompassing a common ancestor and its lineal descendants, branching out over evolutionary time. In this view, individual organisms are not *instances* of a species, but rather comprise "one physical part of a large scattered object," situated in an evolutionary process (Godfrey-Smith, 2013: 108).

Molecular parasitologists at the SLRC estimate the deep histories of heritable materials, like nucleotides and their associated proteins, by building evolutionary models of relationships between sequences on their computers. The extrapolative task of comparing the complex structure of novel genes or proteins with known sequences from other organisms, presents a statistical and mathematical problem that can only be solved digitally.[1] Similarities between two conserved sequences may

indicate shared ancestry ("homology") and can reveal clues about potential entanglements in crucial biological mechanisms. It thereby helps to narrow the search space for suitable RNAi targets.[2] While manual examination of short strings of sequence is theoretically possible, and was common before the dawn of bioinformatics, the gene and protein sequences relevant for contemporary bioscience are now too multitudinous to be meaningfully compared this way. Today, they are primarily stored and analyzed in an automated fashion, as their one-dimensionality and symbolic structure make them tractable for computational and statistical procedures (Stevens, 2013: 41). On basis of shared characteristics, genes, and proteins can be organized into structural and functional groups by evolutionary descent, like "families," "multi-gene families," and "superfamilies" (which may contain hundreds of genes or proteins).[3]

Phylogenetic thinking is mediated through software based on mathematical algorithms for handling strings of nucleic or amino acids. These facilitate comparative analyzes of sequences stored in online databases, freely available to anyone with an internet connection. Such tools both represent evolutionary relationships of sequence, and function as critical infrastructures for data management. A sequence alignment, for instance, usually contains a list of species (from which relevant sequences are sampled using a database), and a long string of letters signifying the respective nucleotides in the case of a gene (or amino acids in the case of a protein). A computer program is then used to identify the best alignments between sequences and highlight salient positions, differentiating them by colors and other representational modalities. From these computer-aided comparisons, biologists gain a better understanding of the evolutionary history, functional expression, and developmental timing of lice genes. Like the sequences they contain, these digital tools have been continuously evolving since Margaret Dayhoff pioneered the collection and sorting of protein sequences in the 1960s. Consequentially, the computer infrastructures that render genomes visible are now something more than saturated repositories of data. Instead of mere catalogues, the way information gets linked in genomic databases embody biological theories and classificatory systems that describe historical interactions between the building blocks of life (Stevens, 2013: 168–169).

Phylogenies are tree-shaped representations consisting of nodes connected by branches. A tree diagram that represents sequences sampled from different organisms should be read as a hypothesis about the ancestral relationship between them, based on a specific model of molecular evolution. Estimating phylogenetic relatedness used to be a hard problem, due to the great many possible relationships that must be searched to fit the data, even for quite small trees. Phenomena like convergent and parallel evolution, as well as evolutionary reversals, homoplasy, massively complicate the estimation of evolutionary relations between sequences. Organisms may, for example, share traits that common ancestors lack. Crafting phylogenies, then, is about determining the best overall fit to the data, given that some data will inevitably fit poorly.

A thorough exposition of how statistical tools are applied in the practice of phylogenetic inference would quickly take us too far afield, but some basic principles are needed for making sense of how computers facilitate "tree thinking" that inform RNAi trials. Phylogenetic relations are best estimates of historical relations, reconstructed through either distance-based methods that compute pairwise distances from sequence, or sequence-based methods that use the sequence alignment to determine the structure of the phylogenetic tree based on an optimality criterion. In my observations of phylogenetic work at the SLRC, my associates preferred a class of sequence-based methods known as "Bayesian inference." "Bayes' theorem" provides a formal framework for incorporating prior evidence (priors) to estimate the probability that an event occurs,[4] and Bayesian inference is part of a family of "character-based methods" that compare all sequences in an alignment by calculating one site in the alignment against others.

To create these approximations, sequences were first sampled from lice and a diverse range of other organisms stored in curated databases, through a BLAST search.[5] BLAST is a collection of programs that can identify and compare regions of similarity among sequences and calculate the statistical significance of matches between them. The most important outputs of BLAST are defined through a "score" and an "E-value," which gives a quantitative estimate of similarity between the input sequence used for the search, and those pulled from the database.

A high score means there are many similarities between the sequences, which may be an indication of their biological relevance. The "E-value" on the other hand is a statistic that reveals the number of alignments that may be expected by chance, such that a lower E-value indicates a "better" hit. Salmon lice gene sequences (or amino-acid alignments) were usually obtained locally through LiceBase before it went public via the online genome browser Ensembl in 2015, or through targeted sequencing of specific genes of interest (when entries stored in LiceBase were inadequate).

Regions of interest were then aligned with genes from other organisms on the computer, using a Multiple Sequence Alignment (MSA) program, such as BioEDIT, and managed with software like Mesquite or MacVector to create a file in the format known as NEXUS. My interlocutors would then import this file into software packages that could run a "model test" to automatically detect the best fit between parameters and models of evolution for the given sequences of interest (such as ProtTest, for comparing amino acid sequences in protein evolution). Next, the challenge was running an evolutionary simulation, with software tools like "Mr. Bayes," or "BEAST.[6]" Here, researchers would choose the preferred parameters of the model for molecular evolution to be applied. While it is possible to get radically different trees as output based on identical sequence data, simply by changing these software parameters, my interlocutors frequently exchanged recipes and templates that specified relevant assumptions for their phylogenies, as these details ranged from the familiar to the arcane. After controlling all relevant parameters, including the number of generations to run (e.g., "two runs for a million generations"), researchers would then execute the phylogenetic inference on their dataset.

This Bayesian process, which evaluates the probability and degree to which a chosen evolutionary model fits with observed data, could sometimes take a day or more, depending on the number of sequences being compared and the available computing power. First, the procedure created a value known as the "posterior probability" by modelling the evolutionary process. This probability depends on what the user is willing to accept as true before initiating the analysis. But due to the number of possible trees, branching lengths, and other parameters, the application

of Bayesian methods alone to phylogenetics quickly leads to insurmountable analytical problems. Phylogenetics must therefore incorporate sophisticated algorithms known as Markov Chain Monte Carlo-models (MCMC) to compute Bayesian probabilities. Crudely put, the Markov Chain is a mathematical system that can model phenomena that jump between different states, while a Monte Carlo simulation is a way to sample random numbers (as in roulette) to simulate stochastic processes that are "too complex to calculate in full analytic glory" (Galison, 1997: 689–90).

A popular textbook for biologists by Wiley and Liebermann, explains the principle of MCMC in the following terms (2011: 223–224): "In general, MCMC involves using computer-generated random numbers and a set of rules to simulate a walk through the space of trees and parameters. One begins by either randomly picking a model (random tree topology and other associated parameters) or by picking a particular model (one considered a priori probable, usually a particular tree topology and associated parameters). One then randomly picks a second model and compares it to the first. If the proposed model has a higher posterior probability density, then adopt it and pick another random likelihood model to test. But if the proposed model has a lower posterior, then it can still be picked with some probability (the probability is simply the ratio of the posterior for the proposed state to that of the current state)."

Commonly, the pedagogic metaphor of hill-climbing is invoked to explain how a distribution is sampled from the evolutionary landscape. Here, one imagines a random process of "walking." Future events only depend on the current state of the process, and not what has occurred before. Each sampled point in the probability distribution depends on the most recently sampled point. First, the "walk" starts at a random point, and then makes a random move. Next, a "height-ratio" between the new and old state is calculated. If the ratio is higher than the value 1, the new state is accepted. If the ratio is lower than 1, a new state is sometimes accepted with the probability of the ratio. If a new state is not accepted, the process stays in the old state. When my interlocutors ran this computerized process for a sufficiently long period, usually for thousands of generations, the simulation would "travel" over this landscape

and approximate the posterior probability of all possible phylogenetic trees.

In practice, these "runs" were a two-stage process. First, there was a "burn-in" period where the program did a heuristic search to find a starting point for the analysis by throwing away some iterations of the MCMC procedure, for instance, the first 10%. Second, there was a "stationary" period where the program explored the parameter space. After finishing the MCMC-runs, the output would then be imported into software, such as TreeView or Figtree, for further analysis and phylogenetic visualization. Representations of phylogenetic trees, so-called cladograms, have a unique branching structure (a topology), containing information about the proximity of evolutionary relations between the represented entities. These are based on a Principle of Parsimony, where the simpler account is usually preferred. It is also possible to draw the same cladogram using different topologies. While some find it easier to assess the relative branching lengths of phylogenies with rectangular tree formats, the use of radial or curved formats is not uncommon, and the choice of cladogram format greatly depends on the representation's communicative and epistemic function.

A tree branch is conceptualized as a lineage evolving through time, and the nodes (the intersections between lines) represent the birth of a new lineage. In molecular phylogenetics, nodes represent gene or protein families and refer to duplication events or may constitute speciation events in cases where tree diagrams are used to represent species relations. By convention, the roots of trees represent the most recent common ancestor of all the taxa in that tree, the most ancient point in evolutionary time. It is also possible to embed information in phylogenetic trees by other means. A longer branch, for example, implies more genetic change, as measured in terms of nucleotide or amino acid substitutions per site. Usually, my interlocutors would annotate their trees with a legend containing a scale bar and a caption that identified this number. Depending on their use, tree diagrams could also be annotated with color-coding schemes that distinguished sequences in *L. salmonis* from other relevant organisms in the sub-phylum Crustacea (like *Caligus rogercresseyi*), or salient creatures like blood-feeders and model organisms.

Because the information used to guide the analysis deep into evolutionary time comes from genetic sequences in contemporary taxa, there was also considerable uncertainty attached to phylogenies. It is therefore necessary to estimate the confidence that a given cladogram reflects "real" evolutionary relationships, using mathematical tools. This meant that for a given tree, confidence in the respective branches could be represented with percentage values for cases where certainty in the branches was less than a 100%.

Researchers at the Centre also had other resources at their disposal to chart and predict genetic pathways of interesting genes before RNAi experiments, such as KEGG (the Kyoto Encyclopedia of Genes and Genomes, see https://www.genome.jp/kegg/). This tool makes it possible to model gene expression profiles and learn about potential gene targets by creating an interactive graphical wiring diagram, composed of hyperlinked representations, that visualize cascades of gene and protein interactions based on information stored in the KEGG PATHWAY Database. In sum, these representations could be used both as a basis for decision-making about further experimentation, and as supportive materials when disseminating experimental results to the larger scientific community.

Use of sophisticated mathematical tools from molecular phylogenetics instantiate what Humphreys calls "epistemic enhancers" (2004). Just like scientists have expanded their sensory apparatus with microscopes, binoculars, and telescopes, so have they expanded *computability* through the discovery and use of new mathematical relationships for learning about evolutionary linkages between genes across taxa. As bioinformatics faces a "quantity of data issue," or a "data deluge" (Strasser, 2012: 85), sequence comparisons have become a far too complex task to eyeball without sensory augmentation. Such analyzes therefore require "property detectors" that can determine the character of specific sequences and their relationships with each other (Humphreys, 2004: 28). Through computer simulations of evolutionary process using statistical models to handle data that are intractable for individuals with their "bare" minds, bioinformatic tools help biologists to extend the reach of their cognitive powers far back into the deep evolutionary past. This constitutes a form of perceptual enhancement through technology that supplements

mathematical skills with computation in ways that boost their cognitive powers.

Bioinformatic tools also increased the speed by which mathematics could be performed and expanded the complexity of problem-solving at the SLRC. In Humphreys terminology, this was achieved both through an "extrapolation" of senses, similar to how telescopes and microscopes aid perception of what cannot be seen with a naked eye, and through "conversion" between sensory modalities, akin to how a visual display can be attached to a sonar to convert soundwaves into a visual representation. Simple structures of short sequences of nucleotides and amino acids are, in principle, available for manual inspection, but the complex sequences of interest to my interlocutors could only be meaningfully compared with computational support. The results of such numerical comparisons can then be accessed in different representational modalities, and converted into a variety of graphical forms that enhanced legibility and support meaning construction (Humphreys, 2004: 4). Additionally, these bioinformatic systems afford what Humphreys calls "augmentation," since no chemical properties of nucleic or amino acids detected through sequencing methods naturally affect human sense organs without some transformation by technological means.

Like many other kinds of computer simulations, Bayesian analysis and Markov Chain Monte Carlo, were black-boxed and not open to direct inspection and verification by most users at the SLRC. This entailed a degree of "epistemic opacity" (Humphreys, 2004: 148). Such opacity was partly an outcome of the underlying mathematical processes, which required special expertise to be meaningful, and partly a result of the software not presenting its users with transparent information about all the stages of the computational process it performed. Rather, the use of phylogenetic instruments was based on practitioners trusting that there were members in the scientific community at large who possessed the necessary conceptual resources to verify what the apparatus accomplished and were familiar with the underlying mathematical principles and biological theory. Expert computational biologists within the practice community thus afforded non-experts with a set of dispositional beliefs that could be consulted when necessary to solve problems and give meaningful accounts of how these scientific instruments operated.

This "dispositional" function was also filled by a wide range of bioinformatic forums, journal articles, discussion groups, and user manuals on the web.

From the perspective of distributed cognition, the representational outputs of phylogenetic inference, like cladograms, summarized complex information about a dynamical process and afforded users with an inductive framework for drawing inferences beyond available information. Furthermore, phylogenies helped to attenuate reductionist thinking about the contribution of specific genes in biological processes. With respect to basic mechanisms involved in gene expression, Jaques Monod famously quipped that what is true for *E. coli* is also true for the elephant. Phylogenetic analysis helped corroborate where such reductionist logics were judicious or spurious.

Beyond their uses in identifying and selecting relevant target genes in the preparatory phases of RNAi experimentation, phylogenies were also useful to contextualize the functional characteristics of select genes in evolutionary terms, as indicated by the previous examples of Receptor R and Protein P. Phylogenetic accounts were therefore commonly included in journal publications, independently of whether these methods had been decisive for selecting the particular candidate genes characterized through RNAi experiments. But although bioinformatic tools were valuable for identifying relevant genes and key biological processes, it was not possible to simulate their empirical outcomes in salmon lice development in silico. Acquisition of robust knowledge of gene expression patterns, and its impact on the phenotypical development of lice, necessitated benchtop experimentation using RNAi in the wet lab.

Final Preparations

Let us now return to The Checklist. After deciding on a target sequence of interest, either through phylogenetic or other means, lab members are instructed by The Checklist to notify the coordinator for RNAi trials via email to schedule participation in an upcoming experimental event. Having notified the coordinator and provided essential information about the fragment of interest, like sequence data and primer positions,

participants would receive a confirmed slot in the queue. Essential information about the gene target was entered into a shared file containing a schedule for when different fragments were due for testing, which was hosted on a server accessible for all members at the Centre. Such queue systems for coordinating RNAi trials were necessary to fully utilize the finite capacity of the experimental facilities. The capacity of the Centre's single-tank system, for example, was often strained due to ongoing RNAi experiments, testing of feed compounds and vaccine candidates, limited because of wanting experimental animals from the proper life stage, or undergoing maintenance. Since sequence data travels easily in the age of computational biology, information that could point toward potential therapeutic breakthroughs were handled confidentially at this stage, due to the proprietary claims of the Centre's industrial partners.

The Checklist refers to RNAi trials using the injection method as "group experiments" for two reasons. First, they were collective endeavors since data from the experimental control group were usually shared between participants to facilitate statistically sound analyzes and reduce the number of fish and tanks spent on each trial.[7] A consequence of this joint arrangement was that experiments could not be terminated earlier or later than 40 days post-infection, unless there was no need for control animals. The rationale was that gene expression measurements from lice in both the control group and experimental condition had to be developmentally synchronized for the data to be comparable. While pre-adult II females were the default life stage according to protocol, experimentalists could also introduce changes, such as experimenting on male specimens or other life stages, if they had reasons to believe this would yield interesting outputs. Such modifications to procedure, however, required additional planning.

The second sense in which RNAi experiments were group-level performances, was that everyone who had candidate genes at stake in the trial was expected to contribute to its practical execution. Before the day of injection, the Coordinator would plan this in detail, such as the hands-on division of labor among participants who had signed on. According to The Checklist, this workforce should be composed of at least four persons, two to facilitate work in the wet lab, and usually a few post-docs, doctoral candidates, or master students, in addition to a

supervising research scientist. Senior scientists, on the other hand, usually took a more active role in the planning and selection of experimental targets, occasionally submitting interesting gene fragments for testing, and regularly contributing to data analysis after termination of the trial.

At this point in the chain of events, the Coordinator would also place an order to the technicians in LiceLab to ensure that an adequate number of lice at the correct developmental stage was ready for experimentation. Participants with a stake in the trial, also had to order primers from online suppliers and perform double-stranded RNA synthesis on the relevant fragments. These fragments were then diluted with a bromophenol blue solution. This solution, as we shall see in a moment, functioned as a colorant that provided a visual indicator that the RNA had been correctly injected into the parasite body. Furthermore, The Checklist also specified that filter papers had to be prepared in advance, to keep lice properly moist during handling. A seemingly mundane reminder, this matter was epistemically significant, since the parasite could be damaged from dehydration if left unattended on the lab bench for too long. A critical loss of lice caused by undetected dehydration at the stage of injection could wreak havoc on the interpretation of gene expression analysis downstream in the pipeline. Ideally, participants also had to prepare a list of prioritized targets, in case there were insufficient amounts of available lice on injection day. Finally, glass needles for the micro injector had to be pulled and sharpened.[8] The latter task was usually performed by the senior laboratory engineer, who also carried out manual injections at the Centre.

In cognitive terms, all this preparative activity of arranging resources in advance of the experiment are instances of "pre-computations" (Hutchins, 1995: 165). In the context of experimentation, pre-computations transform the nature of epistemic tasks and activities, as the performance of actions in the past redistribute workload across future events. In this case, pre-computations fundamentally change the informational environment of experimentalists by setting up novel structure in their task environments ahead, that help them perform time-sensitive tasks. By carrying out essential calculations and projections in advance, and embodying the outputs of these in representational artifacts, experimentalists can solve certain epistemic tasks using only simple perceptual

inferences and manipulations of material structure during the actual time-limited execution of the RNAi trial. Pre-computations thereby transform the experimental environment by capturing invariant properties pertaining to its design and activity-structure in physical artifacts, including representations of procedures, and arrangement of resources in physical space. The basic set-up in different experiments, for example, ought to vary as little as possible between different trials to produce reliable results, and experimentalists secured comparability of outcomes by managing instruments and ingredients in advance. With Hutchins, we can see these pre-computations as a window onto experimental practice as an extensive cultural process (1995: 168). In this case, a cumulative, material culture that has collected a plethora of representational modalities to help practitioners solve frequently encountered problems in a functional environment for knowledge-making (Fig. 5.1).

Injection day

The most conspicuous event spelled out in The Checklist is the "day of injection." Here, participants in the RNAi experiment are instructed to arrive in the lab early in the morning, since the procedure requires an entire workday to complete. Let us now look closely at an ethnographic vignette from an RNAi event at the Sea Lice Research Centre to understand how interactions between material, social, and cognitive resources for executing the experiment were managed.

Standing with our backs toward the entrance in a wet lab at the Institute of Marine Research, we are facing a narrow, brightly lit room that is tailored for the task at hand. There is a workstation to the left filled with various technical equipment: forceps, petri dishes, plastic bottles and containers in odd shapes and sizes, stacked boxes with disposable gloves, a wash station, and an under-the-counter dishwasher. A heavy, red plastic curtain bundled together with a piece of rope, divides the room in two. It marks off a separate "clean-space" for microscopy, computer work, and other delicate benchtop operations, like the micro-injections that will soon take place. On the right side, midway along the wall, is a door leading into a larger room, filled with rows of single-fish tanks

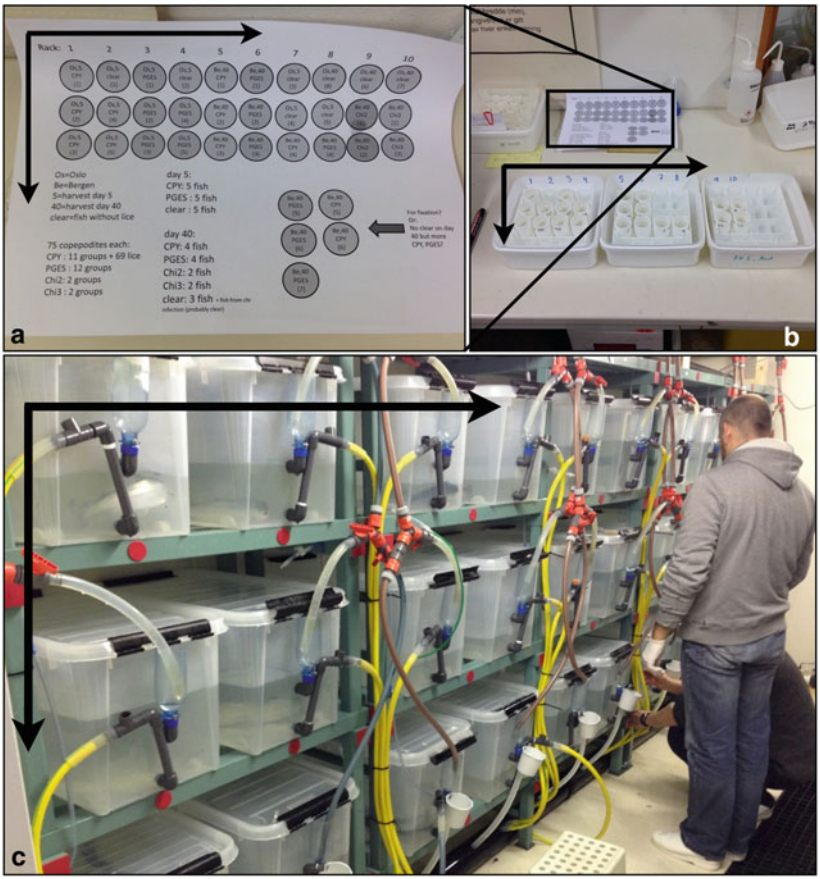

Fig. 5.1 Pre-computations in an RNAi trial. **a** Spreadsheets highlight the stabilizing role of pre-computations. Template and physical array of hatching wells **b** and single tanks **c** are set up in advance, providing mutually supporting structure to maintain order in biological materials and stabilizes enacted representations. "Trajector-based" cultural practices, and ecological assemblies, figure prominently (see Chapter 6). This experiment was executed in LiceLab in November 2013, in collaboration with an Oslo-based Work Package to study gene expression in interactions between salmon hosts and the parasite. One group analyzed gene expression data from lice, the other focused on the salmonid immune response. Genes relevant for the former are labelled BE, while those of interest for the latter are labelled OS. Each column represents an experimental condition. Top row, corresponding to tanks in rack 2, 4, 7, 8, 9, and 10 are labelled "clear" (fish without lice). CPY refers to the control fragment (see Chapter 6 for details)

stacked in columns containing juvenile salmon. The single-tank array inside this room is almost identical to the one we encountered earlier, in the basement wet lab at the High-technology Centre. The similarities between the two configurations are not coincidental. After all, this wet lab was designed and assembled by the same people now responsible for managing the wet lab at the University, during their previous tenure at the Institute. Now, the wet lab was tended by another group of technicians, who had inherited this habitat, adding their own modifications and routines to it. On this morning, the team responsible for injecting lice with double-stranded RNA and reinfecting fish included:

- Ada, a chief technician with extensive experience with RNAi trials. In the words of one PhD candidate she "knows everything" about the lab.
- Veronica, a doctoral student working on fibronectin type II-domains.
- Lena, a postdoctoral candidate working on aspects of the chemosensory system of salmon lice.
- Sara, Veronica's supervisor and one of the scientists who were instrumental in adapting RNAi technology for salmon lice. Sara coordinates the large-scale RNAi trials at the SLRC.
- Robert, the engineer responsible for the wet lab, and two other technicians who will tend the fish.
- The ethnographer.

The first task on the agenda was to tranquillize the salmon and carefully collect the pre-adult salmon lice using forceps. Afterward, the lice would be injected with synthetic, double-stranded RNA using a manual microinjector, upon which the modified specimens would be left to incubate, and then reinfected to new salmon hosts. On this day, Sara had a busy schedule, and had to supervise the experimental processes from her office on a different floor in the building, intermittently dropping by the wet lab to see how things were progressing. Accomplishing all the necessary tasks for a successful trial demanded a complex coordination of both people and things, so Sara had assigned specific duties to everyone upon convening in the lab. Additionally, participants were expected to assist with any time-limited tasks that arose from the activity stream.

At first, some of the participants were unsure about their designated role within the experimental choreography. But it did not take more than a few minutes before the group had distributed responsibilities underspecified by the Coordinator's instructions and settled into a pace that moved the work forward. As the researchers were busy preparing the wet lab for injections, Robert and two other technicians worked outside the main building in the December cold. Here, they harvested lice from the large salmons that were housed in the communal fish tanks occupying the Institute's courtyard. Geared up with headlights, gloves, and insulated boiler suits, the outdoor team used forceps to delicately remove salmon lice from sedated fish and aligned them in a 5 by 2 grid, on a moist wet paper in plastic petri-dishes. Each dish contained 10 females and 10 male pre-adult lice, which were then carried by a runner (and occasionally the ethnographer) to the team inside the building, who were responsible for organizing the actual injections.

Picking lice is a delicate, and by no means trivial, step in the execution of RNAi injections. As The Checklist underscores, mismanaging this step could have epistemic consequences: "Be careful when you handle the animals, avoid pulling or poking the genital segment and the abdomen. Make sure that the forceps you are using is in good shape. This can be time consuming if you are inexperienced, but remember that the lice prefer the environment to be cold and wet." Both fish and lice are fragile, and injuries on the experimental organisms at this stage could potentially introduce noise to data procured from the system, thereby threatening the veracity of subsequent analyzes. Although such knowledge was seldomly made explicit in external communications about experimental results, Kohler reminds us that craft skills of this kind have been essential to progress in the history of experimental biology (1994).

Microinjections

When the lice pickers in the courtyard outside had sampled ten pre-adult lice and arranged them on a wet paper placed in a Petri dish, this batch was swiftly brought inside to the benchtop where Ada sat in front of the stereomicroscope, ready to inject the specimens. To accomplish this task, the chief technician applied a micro-injector, driven pneumatically by manually controlling a mouthpiece. This exemplify what Hacking calls a "source of modification" (1992: 46), the part of the apparatus that actually interferes with the epistemic target. As such, the research group had made considerable efforts to fine-tune the injection technique, which had been a bottleneck for delivering double-stranded RNA to silence genes in lice.

Normally, an average of thirty lice were injected per gene fragment. The mouthpiece itself was connected to a plastic tube, approximately one meter long, which was casually slung across the technician's upper back to keep it out of the way from her dexterous hands as they worked swiftly in concert to expedite the parasite, one by one. To use the injector, the mouthpiece was first inserted between the lips, while the glass needle was held in the main hand. Then, a small amount of synthetic, double-stranded RNA, tailored to the genetic sequence of interest, was drawn into the needle from a test tube with the help of capillary action. At this point, the other hand introduced the forceps and positioned the louse specimen on the Petri dish below the stereomicroscope's objective, while gently keeping the animal steady. Looking through the eyepiece, the glass needle was then carefully guided toward the cuticle of the dorsal region, and once positioned there, aligned with a distinct location on the parasite's back, where the exoskeleton forms a natural segment which conveniently afforded insertion of the glass needle. Squinting through the ocular lens, the injector had to carefully guide the glass needle into the segmented area and insert it below the cuticle plate, while holding the lice steady. With the needle "in place," a verdict based on proprioceptive feedback from the tissue and visible confirmation from the stereomicroscope, the experimenter would then gently blow into the mouthpiece, pushing the solution of dsRNA and colorant into the organism.

As explained by Ada, this was the tricky part, since the fluid's viscosity was not homogenous and could therefore clot the thin needle. Blowing too hard could result in too much fluid being injected into the parasite, which not only made a mess, but could potentially kill it. On the other hand, if air was pushed through the tube too cautiously, the technician might fail to introduce sufficient double-stranded RNA into the parasite, and thus fail to get the desired interference response. Meanwhile, there was also a constant risk of crushing the specimen with the forceps, skewering it on the glass needle, or otherwise damaging it through careless handling. Participants therefore ensured that they did not disturb Ada's delicate work. After being dispatched in the animal, the bromophenol blue staining would yield instant visual feedback that the RNA was properly injected. If the colorant started bleeding excessively this could indicate that the procedure had missed its target and damaged the specimen.

To the extent that it was practically feasible, Ada conducted all injections for RNAi trials at the Centre. When I asked why this was so, her colleagues emphasized her dexterousness and experience, recognizing that she had simply acquired more tacit knowledge about the procedure than the others in the group. Additionally, there was an epistemic motivation for why she performed the job. I was told that, methodologically speaking, it was preferable that the same individual who injected the control fragments was also the one responsible for injecting fragments across experimental conditions. Since every member of the laboratory was assumed to hold idiosyncratic mannerisms that could influence the execution of injections and impact the experimental outcomes. It was better if one, reliable colleague conducted the injections, thereby minimizing variations within and across experiments to the largest extent possible.[9] This set up a positive feedback cycle, as Ada acquired more experience and proficiency with the task than others at the Centre. The drawback was that expertise in a crucial skill for the experimental system was concentrated in one highly entrained individual, as other lab members would require much training to accomplish the procedure as reliably and proficiently as Ada.

At this stage, it was crucial that researchers duly kept track of their samples, along with any inscriptions that were to accompany these

further down the experimental pipeline. For example, on the bench next to where Ada performed her injections, several square plastic containers were organized in a grid on the counter, next to a stack of additional wet papers. After each batch of lice were injected, the wet papers were then picked up by another participant and transferred to a plastic container filled with seawater, which carried a small note inscribed with the specific fragment number that had been injected into the lice, before it was left to incubate for several hours. In all caps, The Checklist reminded participants about the gravity of keeping track of representations as they propagated throughout the experimental system: "IT IS ESSENTIAL TO KEEP TRACK OF WHICH LICE GOES IN WHICH INCUBATOR, LABEL CLEARLY WITH FRAGMENT NAME." This simple act of marking significant content with inscriptions stabilized the relation between thoughts, acts, and manufactures. Following this pattern, injections would usually continue until all fragments were expedited, so that when the group started working around 8:30 in the morning, they could have their lunch around noon. The lice would then be left to recover for three hours. Specimens had to be well-rested so that the parasite could again latch onto a host fish during reinfections (Fig. 5.2).

Reinfection

After lunch, the group reconvened in the wet lab to place the RNA-treated lice back onto new salmon specimens. These fish first had to be anesthetized by the technician, which presented yet another bottleneck. As with lice, the fish had to be carefully handled to ensure adequate experimental results. Over time, the Centre had acquired routines for optimizing the drug combination and dosage time used for anaesthetization.[10] If the fish spent too little time in the sedative solution it would flap around violently, and its handlers could injure the fish, or themselves, on forceps and other sharp equipment. Too much anesthetic, on the other hand, could kill the prized experimental fish.

While fish were prepared in the adjacent single-tank storage facility, the scientists got busy collecting individual lice from the plastic hatching

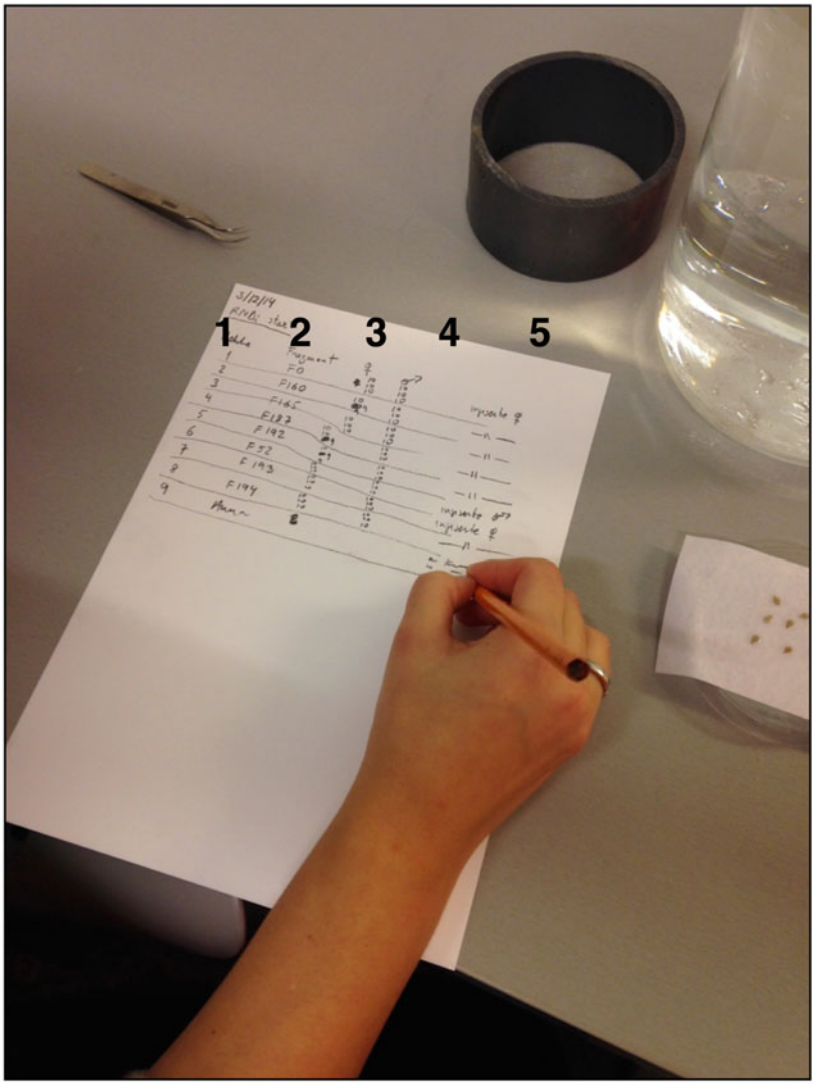

Fig. 5.2 Tracking experimentally modified salmon lice and RNA fragments. **1** Leftmost column contains the single-tank racks from 1 to 9. **2** The next column holds the fragment names. A running number (Fxxx) refers to a list of screened candidates. Column **3** and **4** lists the number of male and female lice for each fish. **5** Lists the sex of salmon lice injected with RNAi

wells they had been placed inside before lunch. Carefully, each louse was positioned on its back on a square piece of paper, using forceps or a gloved finger. The fish was then placed on the paper, which was labelled with a fragment number. Alternatively, the paper was aligned across the fish skin, and then gently pressed against it. The theory behind this maneuver was that the parasite would engage its chemo- and mechanosensory apparatus when coming into proximity with the salmon and then latch on to the skin. Lice are attached to salmon via a frontal filament during the chalimus stage, but at the mobile stages the parasite uses its smaller extremities and gains help from a body plan evolved to keep it tightly attached onto the surface of a swimming host. The wet paper was then removed, and the fish carefully reinserted in one of the single tanks. Single-tank arrays were designed so that three fish tanks in each experimental condition formed a stacked column, with each column sharing the same water supply and outlet, thereby marking of a separate experimental group. Information about the exact fragment that had been injected into the lice on a given salmon, as well as the coordinates of its tank (a letter/number combination) were then logged on a piece of paper which was subsequently plotted into an Excel spreadsheet. These routines, and the symbolic conventions that governed the experimental ingredients, were in continuous development to improve the system's determinacy and stability.[11] When all the fish had been infected and returned to their tanks, it was time to tidy up the lab, and clean the workbenches. It would take more than forty days before the outcome of this material remaking of the world could be revealed.

Running the Experiment

Clearly, RNAi screenings were concerned with more than the mere transformation of biological structures, as these epistemic events also created, propagated, and transformed representations through a variety of media, thereby setting up vital relations that supported the cognitive life of things in the laboratory.

As The Checklist instructs: "for each experiment there will be an Excel-sheet that need to be filled out for every sample taken during and at

termination of experiment." Every RNAi screen conducted at the Centre was linked to a running number, and individual samples were given a unique code in the order which the samples were taken. This information was then integrated in a digital spreadsheet that converted every sample into a trackable representation of salient events within the experimental system. These encodings provided a collective, external memory of how the phenomenal objects of interest moved through the pipeline of the experimental system. They also afforded a simple way to transform the representational states of each experimental event, as it moved through various *in vivo, in vitro*, and even in silico systems for handling biological data. For the latter purpose, "The RNAi Experiment Annotation Checklist" specified in detail the correct procedure for curating experiments in LiceBase, outlining two sub-checklists. The first was to be completed when an RNAi experiment was initiated, and included a free text summary, a general entry on metadata such as the Batch ID, date and contact information, as well as a description of the sample, the gene target, and the RNAi fragment. The second sub-list was to be followed when the experiment was terminated and included information about the efficacy of gene silencing, detailed accounts of the resulting phenotype, and relevant image files.

Scientists tracked the progression of their RNAi experiments during the prescribed forty-day period by visually inspecting lice in the tanks, as they were attached to the fish. During this phase, they would look for signs suggestive of whether there was a silencing response working on the targeted genes in the organism. But there was no general rule that articulated what they ought to look for. As The Checklist underscored: "The level of monitoring that you will perform from now on until termination of the experiments depend on YOUR experiment." The kind of observations that were relevant was contingent on the genetic pathway and the biological phenomenon under study. Delayed maturation, for example, could indicate a strong interference response in cases when RNAi aimed to silence genes related to developmental processes or reproductive functions. A critical lice loss in the post-infection period was another indication of an effective RNAi knockdown, but whether this was signal, or noise, was circumstantial. The Checklist specified: "We normally see an unspecific loss of lice, the first couple of days. These

are probably lice dying from handling damages. After that we have to consider mortality as a possible effect of the knock down." Instances of "unspecific lice loss," had an ambiguous epistemic status, and it was difficult to attribute the direct causes of such observations. RNAi-treated lice, for instance, could sometimes vanish into the water drain without trace. This latter problem could partly be mitigated by placing small nets on the outlet, but since biological debris like fish feed and mucus accumulated on the filters, they needed frequent inspection. On other occasions, free-floating parasites were eaten by their host. Poor infection rates by lice on the fish could also be a consequence of rough handling during the injection phase, and intermittently there were disconcerting interactions between the salmon and their tank environment, such as rubbing against the plastic walls. All these events could produce unspecific losses, so despite concerns over experimental control being a key motivating factor behind the move from collective fish tanks to the novel single-tank system, it was next to impossible to eliminate every potential confound when studying host–parasite interactions.

Termination

According to The Checklist, RNAi experiments are usually terminated after 40 days, which ensure that a second generation of egg-strings have developed on the female specimens. The main agenda for termination-events was the removal of lice and egg-strings from the fish hosts (both experimentally treated parasites, as well as the control group), and preparations of lice tissue for the physical, biochemical, and representational transformations that followed. In the end, these transformations would result in measurements of gene expression. By integrating such informational structures, scientists created meaningful accounts of the molecular characteristics of gene function, revealing new clues about potential therapeutic applications.

Like in the injection phase, the termination and handling of biological materials from RNAi-treated salmon lice, required participants to carry out a variety of new pre-computations. The Checklist instructed experimentalists to prepare stereomicroscopes and cameras, and to add

chemicals to small plastic test tubes that preserved lice for both tissue-sectioning and gene expression analysis. Preserving samples that would undergo anatomical study using the microscope or other imaging techniques, was achieved with "Karnovsky's Fix." A fixative substance is made up of molecules that easily form cross-linkages with biological targets, enabling the preservation of whole tissues.[12] Other tubes were filled with a substance known as "RNAlater." While Karnovsky's preserve whole pieces of tissue for visual inspection, RNAlater is a storage agent that conserves fragile RNA for molecular analysis.[13] Without RNAlater, the scientists would have to immediately process their samples, or freeze them in liquid nitrogen, which would entail a cumbersome process of grounding and homogenization, with constant risk of thawing the precious tissues and thereby compromising the valuable information carried by its molecular configuration. By placing samples in RNAlater, these could instead be stored for a month or longer, in the refrigerator or long-term at below minus 20°C, until there was time to transform and analyze the material, beginning with a biochemical procedure known as "RNA-extraction." In the next chapter, we learn how such materials are handled by scientists downstream, in order to learn more about gene expression profiles of experimental candidates.

Among the final preparations before the day of termination was a mundane, but critical, task that involved printing out a series of sticker tags. These labels carried the date of the experiment, its name, and sample IDs. Stickers were then placed on the tubes containing Karnovsky's and RNAlater. They could also be attached to paper sheets with inscriptions about what was to be observed. By attaching these on the hatching incubators used to rear the new egg-strings, it was possible to track the contents through further processing. A hatching sheet for logging lice numbers was also printed out beforehand, to afford easy inscriptions of relevant details about biological phenomena that materialized during the termination event.

Let us now approach an instance of RNAi termination and look closer at how representations are enacted through the experimental pipeline. By zooming in on minutiae in a video-recorded sample from one such event, we can better grasp the iterative material engagement and socially distributed cognition that sustain "contexts of discovery," and how these

become epistemically important for the generation and justification of experimental knowledge (Schickore & Steinle, 2006).

We are back in the same wet lab from our earlier visit. In this new scene, there are five individuals at work, busily preparing various technical equipment, documents, and biological samples. This time the participants include:

- Sara, the Center's RNAi coordinator.
- Hanna, who is a postdoctoral candidate at the SLRC.
- Greta, an exchange student from a German technical school with a laboratory internship.
- Robert, the wet lab engineer responsible for handling the fish.
- The ethnographer, who again observes and awkwardly participates by helping with simple tasks.

As with the initiation event described before, participation in the termination phase usually occurred on a rotational basis. A limited number of fragments were tested in each trial, and although not everyone who had a candidate fragment at stake in the experiment had to be present, it was expected that some of the graduate students and postdoctoral candidates volunteered to participate.

The main tasks during the termination were delicately removing the experimentally modified salmon lice from the fish (along with any control specimens), registering salient information, photographing each phenotype, tissue preservation for RNA analysis, and sampling lice on fixative for morphological analysis. As a regulatory representation, The Checklist specified a "cultural script" for how this activity should done within the experimental system (Shore, 1995; D'Andrade, 1995). This asserts a set of shared epistemic norms and values circulating in the community. But in addition to any conventions laid down by The Checklist, Sara also provided multiple instructions on the fly, further specifying who should do what, where, when, and in what form. So, while the written script offered a general plan for how the group could organize their experiment well, it was also necessary with additional micromanagement. These instructions pertained to a range of different ecological conditions to ensure the production of high-quality data, such

as the level of experience among the participants, the size of the experiment, and any unforeseen circumstances that might occur. Furthermore, several parts of the activity system remained underdetermined by both The Checklist and Sara's instructions. This meant that certain aspects of the situated action that were not covered by the plans for the termination event had to be determined on the spot, depending on unpredictable contingencies specific to the epistemic situation (Suchman, 2007). These episodes demanded that the experimental actors aligned their resources in novel ways to address the fleeting problems at hand. As the activity unfolded, the global script of the event even faded into the background, as every member of the team came to act only when certain environmental conditions were fulfilled.

Compared with the experiment's initiation phase, termination events required more coordinated work to be performed, and these activities were also more diverse. The nature of cognitive work during such interactions can usefully be understood as "sequentially constrained," to adopt Hutchins' vocabulary (1995: 198). We can say that a task within an activity system is sequentially constrained "if the execution of any enabled operation will disable any other enabled but as yet unexecuted operation." Whether actions are sequentially constrained or unconstrained, depends on both the formal properties of the action structure, it's execution, and how it is represented. Sequentially constrained actions often require hierarchical coordination between different subtasks, although they may, on occasion, also be improvised. In contrast, sequentially unconstrained actions require only loose connections and communications between the involved actors. Let us now see how this occurred in practice to support data production.

At the onset, Sara is seated by the stereomicroscope. Hanna is busy labelling test tubes together with Greta, using preprinted stickers that Ada prepared in advance with a special printer. Hanna and Greta are tagging two different kinds of tubes: Karnovsky's tubes are for tissues that require sectioning, and RNAlater tubes are for RNA extraction. Each tube is given a running number and labeled as either "FIX" or "RNAlater.[14]" A Styrofoam box with crushed ice keeps the tubes temporarily refrigerated on the workbench. Again, we see epistemic pre-configurations of the environment.

Just before the main action is about to start Sara, as the senior authority, explains in English the logic of what is going to happen next. She switches to English due to the presence of Greta, a German lab intern. Here is a simplified transcript:

Sara: Ok, so the idea is that Robert [the wetlab technician in the adjacent room] takes the fish out of the tank and he picks off the lice. And he will put them on a Petri dish and line them up. And one person then brings them in to the people sitting at the microscope. So, there would be one who can take it [points to the microscope], and one person pretty much sits and takes pictures. And the egg-strings have to go into the hatching [wells], and it has one person sit and note everything in Excel-sheets; how many females, and which females are going on RNAlater, and which females are going on to fix.

Ms: How do you decide which goes to fix and which goes to RNAlater?

Sara: As a default there is one animal per tank that goes to fix, but you have to look a bit at them. Like, if there is, if all look the same and there's one that's normal you don't put that on fix, and if there are very few animals, you have to have at least five animals on RNAlater. Cause you cannot do qPCR if there is less than five. So, in that case, we take less on fix. So, it's quite, yeah. There's sort of full-time, one person going to take the pictures and egg-strings off, and one full-time person doing the Excel. And then, maybe Hanna, you do the Excel, and I'll take the pictures. And Greta and you [points to the ethnographer] go back and forth [carrying lice]. And, yeah [goes on to talk to the technician picking lice of the salmon in the adjacent room].

Having communicated her expectations for appropriate future states of the experimental system, Sara went over to the bench and configured the microscope-mounted camera. In the following activity, she would be concerned with three epistemic tasks. The first was to visually inspect the salmon lice and carefully remove the egg-strings with forceps. There is a significant skill component involved in this task. Mature egg-strings,

brownish in appearance, are relatively easy to separate from the genital segment of gravid lice with a gentle pull. But the opaque and fresh egg-strings require more force to separate and can easily be crushed with the forceps. After separation, the egg-strings were to be placed in a hatching well, while the salmon louse is scrutinized for abnormal phenotypical traits resulting from gene silencing. Additionally, Sara must decide for each group whether a sampled tissue should go on FIX or RNAlater for processing.

As Sara made her final preparations, Hanna and Greta marked up the remaining tubes with running numbers. Hanna explains that her task is to log the number of lice, their sex, and the fragment number. She recalls that the last time she participated in this phase of an RNAi experiment, she sat by the microscope while another researcher performed the Excel logging. Then, before the cataloguing begins, Hanna, Greta, and Sara take another moment to coordinate a few last-minute details surrounding the order of work and tasks to be performed. Since this is the first time Hanna registers information in the spreadsheet, she worries about committing errors that may negatively affect the outcome of this collective work. Among the things she is hesitant about is whether each male and female louse is supposed to have a dedicated row in the spreadsheet, or whether they all go in the same one. When Sara starts to pick lice, Hanna should ideally have set up her spreadsheet with an adequate system of inscriptions so that she can quickly enter the relevant information into the cells. But she is unsure about the best way to organize and label the columns and rows. Sara calms Hanna's reservations by assuring her that they will start slowly (Fig. 5.3).

Robert intervenes to inform that one fish has unfortunately died during the experimental run. Since the lice specimens attached to this fish have perished, they can only harvest lice from 23 fish. One of the main challenges for Robert in the coming hours, will be to monitor the anaesthetization of each individual. He must carefully monitor the life signs of each fish by taking observations of its respiratory function. Ideally, it should be sedated for 90 s, which is roughly the time it takes to harvest lice from the proceeding fish. Since the parasite has now matured to the adult life stage, and is perceptually salient, picking them is relatively easy. Originally, the fish was infected with ten pre-adult females

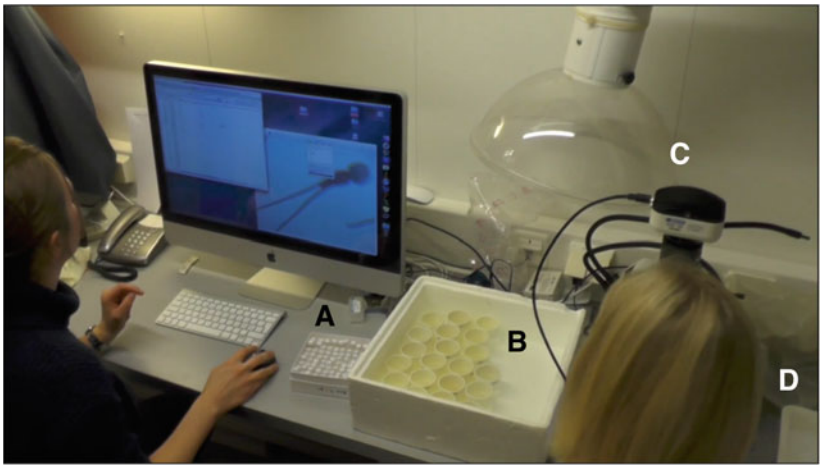

Fig. 5.3 Hanna is seated on the left; Sara sits on the right. The intern who delivers specimens from the technician in the adjacent room enters with fresh specimens from the left (not visible in image). Items on the workspace from left to right are: **A** Box with labelled test tubes with running numbers for fixative which is added under a fume hood later **B** Hatching wells immersed in seawater for the egg-strings. **C** Microscope mount with camera and Petri dish containing lice array. **D** Styrofoam box with ice for tubes containing RNAlater (partly visible white container in lower right corner)

and males. He explains that there are normally five to six lice remaining per fish based on experience, and roughly an even number of males and females. Each single tank has been fitted with a filter in the water outlet, so they can keep track of any lice that have fallen off.

As the harvesting begins, Hanna again expresses concerns about the organization of her task space, and how her activities should fit into the overall flow of action. Adding to the challenge, she also experiences problems with pre-formatting the cells in the Excel document. The lab computer is an Apple iMac, and she usually works on a Windows PC. "I hate Excel!" she frustratingly exclaims at one point, as she uses an unfamiliar keyboard shortcut to prepare the spreadsheet, entering identical dates into a set of columns.

Standing next to the workbench, Sara then gives an instruction to Hanna that they will first receive batches of lice from Rack 1, which contains the control fragment. She explains that each subsequent rack

containing three fish, will have lice injected with the same fragment. This introduces expected regularities, an ebb, and flow of activity. She will later notify Hanna about which fragment is being handled each time Hanna is supposed to enter information into the spreadsheet. The characteristics of each female lice are supposed to be inscribed on a separate row, while information about males is added separately. Generally, male specimens are not converted into critical data, and are regularly discarded at the end. Sara explains that the main function of the male count in this experiment, is to confirm that there were male lice around to impregnate the females. If there are no males present, they must make a small note, in case the female does not develop any egg-strings. Absence of egg-strings may either be explained by lack of mating partners, or by the efficacy of RNA interference. Occasionally, there are situations when males are examined more closely to get comparative data on gene expression and function. One example is research on regulatory differences in gene expression between males and females at various life stages, which offer insights about genes involved in reproduction.

After finalizing the preparations, Hanna and Sara receive their first batch of lice from the intern, who hands them over from the technician. Robert is now busily at work in the adjacent wet lab, anesthetizing fish, picking lice, and arranging them on the Petri dishes, carefully marking each dish with the rack number (for instance 2B), and the number of specimens from each sex next to a small Mars and Venus pictogram. By the bench, Sara reads out the relevant variables for each dish to Hanna, who inputs this information into the computer. When she receives a batch of lice from Greta, Sara calls out the rack/tank number (for instance, "three C"), as well as the numbers of males and females on the dish ("three females, one male"). She also adds additional information, such as: "the first goes onto fix!"

Intermittently, Hanna repeats the values called up by Sara. This serves two purposes. It provides Sara with a chance to correct Hanna if she has misinterpreted Sara's commands. Also, it helps Hanna form a more stable representation of the information that she is entering in the spreadsheet. This way, shadow talk works as a guide to epistemic action. Note also that this verbal interaction is not specified in any instruction and emerges on the spot, generated from the dynamics of the activity. Finally, Sara calls

out the number on the hatching well, where she will put the egg-strings for later studies of fecundity, so that Hanna can inscribe this information into the spreadsheet as well.

During this process, a picture is taken of each louse specimen from the dorsal perspective, looking top-down onto its backside.[15] Since the camera is operated via the computer, this task sequentially constrains Sara and Hanna's actions even further. Before the photo can be taken, Sara must first signal to Hanna that the louse is in focus, exclaiming "der!" ("there"). Upon hearing Sara's signal, Hanna then shoots a picture using the microscope-mounted camera, which is operated by the computer through the keyboard and a mouse. She then saves the picture in a folder dedicated to this unique experiment. The file is inscribed with the running number, and each louse specimen is then placed in tubes that also have been labelled with this same running number. Note here that Sara has access to their joint domain of scrutiny both via the computer screen and the ocular, while Hanna can only access the information on the screen and observe Sara's behavior. She cannot interact directly with the specimens. Sara's commands therefore provide directions for action that both captures salient features of the world, as Hanna makes a written representation in the spreadsheet, and transitions the experimental system into a new state. These utterances are "status reports" (Hazlehurst et al., 2007: 547), that intermittently create shared understandings of the current state of the distributed cognitive system of RNAi termination. Each report also sets up expectations for specific epistemic actions to follow.

Specimens were subsequently placed either on fix, or on RNAlater, and numbered in the order they were collected. This number corresponded to the number on the fish tank from which the lice was harvested. Egg-strings were placed in hatching wells numbered with a letter (designating a set) and a number (an exemplar within the set). This way the maternal identity of the egg-strings became linked to the incubator identity in the spreadsheet (for instance: "egg strings from specimen RN17, goes into hatching well A19").

As specified by The Checklist, the first louse from each tank would be placed on fix, while the remaining lice went into RNAlater (if the experiment produced more than five animals for RNAlater, it was okay to

put one more on fixative). Sara observed that while this was convenient, this pattern was not without epistemic risks, since it could introduce a slight chance of systematic bias into the data. The rule of putting the first specimen on fix would be fine, had it not been for the fact that pickers tended to be slightly biased, and therefore pick out the largest and most visible females from the Petri dish first. They could therefore, in the worst-case scenario, introduce a systematic sampling bias into the experiment that skewed later analyzes of the lice placed on fix. Vigilance about such factors was a consequence of deep familiarity with the experimental system and its biography.

Each movement of specimens and samples between containers was followed by an inscription entered on the spreadsheet. The spreadsheet would subsequently act as another coordination device within the experimental system. It connected a series of inscriptions with specimens on fixative and RNAlater, egg-strings in hatching wells, photographs of experimentally modified lice, and metadata associated with these photos. Deploying Hacking's terminology, we can say that these inscribed "marks" form a critical linkage between the constituent, biological epistemic things that make up the experiment, and the subsequent processing of "data" (1992). With reference to this taxonomy of self-vindicating elements in the lab, the human agent makes decisions and acts as a kind of "data generator", by productively transforming representations of one kind into a different kind. Downstream in the experiment, the various materials being handled, such as lice on FIX and RNAlater, and the egg-strings, will again encounter many other types of data-generators, of both the human and nonhuman variety.

From the perspective of distributed cognition, it is also interesting to observe that the flow of collaborative work on several occasions went out of sync during the operation, but it was also repaired without a plan. Intersubjectivity between the participants required the mutual fulfilment of expectations, but it was sometimes challenging for Hanna to predict what would happen next. Hanna had only partial access to Sara's task domain. Sara could freely inspect both the monitor, the microscope, and had a wealth of available information from her tacit interactions with the specimens at hand. But Hanna was only privy to information about pending actions from the computer monitor and the emergent structure

afforded by Sara's actions, without disrupting her workflow. This asymmetry necessitated intermittent creation of shared understandings and alignments about the current state of the system, particularly by using talk as a coordination device for joint action. On one occasion, Sara asked Hanna whether she had recorded whether they had harvested one or two egg-strings per female lice. When Hanna confirmed that she had not kept track of this information, Sara reassured her that it nonetheless mattered little. The number of egg-strings could instead be read directly off from the digital pictures stored on the computer. There was, fortunately, unplanned redundancies in the system that ensured that a slip-up only negligibly disrupted the downstream informational environment.

Photographing the specimens presented another coordination problem. Pictures of gross salmon lice morphology serves as the first traces of interesting phenotypical changes arising from RNAi exposure. But on several occasions in the sequence above, the egg-strings were simply too long to capture within the camera frame. It was therefore necessary to take two pictures, with an intermittent realignment of the specimens in-between, to satisfactorily document the whole animal. On other occasions, undesired processing anomalies known as artifacts, appeared on the pictures, potentially complicating interpretative work. At one point in the sequence, Sara notified her colleagues that something was wrong with one of the specimens they were working on. She signaled for the group to examine the monitor, then scrutinized the scene for a moment and reported that there were in fact two individuals in the image. It was soon evident for all that a male louse and female louse were mating under the microscope. On yet another occasion, the intern noticed that there was more than one pair of egg-strings in some of the hatching wells. It turned out that remains of old, dried-out egg-strings could be found in several wells. This called for additional problem-solving. Robert was duly notified to double-check the remaining hatching wells and make sure there were no old egg-strings mixed up with the fresh ones, as this could complicate analysis later in the pipeline.

From the distributed perspective, complex multi-agent activities like RNAi experiments are bound to face minor deviations that swiftly demand identification of problems and corrections of action (Hazlehurst

et al., 2007: 547). We can see "alert notifications," like those presented by the agents to each other, as stemming from events that caused a "perceived deviation" from the desired system state. But despite minor setbacks, the team was able to create updated and joint understandings of their shared problem-space, and quickly realign their practices. The meaning of these epistemic events emerged both from the affordances given by pre-configurations of the task scape, and emergent structures of interaction between the agents, such as the bootstrapping process by which Hanna corrected and caught up with Sara's instructions for what to do with the inputs to the spreadsheet. Interactions between these elements of the experimental system thereby offered new constellations of cognitive resources, that helped to order, propagate and transform the representational and biological outputs of the RNAi experiment.

Wrapping up

Just before the session ended, after approximately one hour and forty-five minutes, Sara instructed Hanna to shoot a blank picture with the camera and save the final picture in the folder with the other images, labelling the file as a "scale bar." This image file would contain information about the shutter speed, magnification, and importantly, information about the camera's pixel size (in μm), three variables which remained identical in all the photos taken during the session.

For photomicrographs of biological phenomena to be legible and meaningful for scientists, in article manuscripts, for example, the community needs to know how large the structures on the photos are. This is achieved by placing a small scale bar in the corner of the image, with a caption describing what length the scale bar represents (for instance "30 μm," "30 microns"). Getting the scale bar right, however, requires awareness of a concept known as "binning." This is a computational procedure that facilitates compression of data by combining a cluster of pixels into a single unit. Electronic sensor systems, such as digital cameras, have a signal-to-noise ratio, which says something about its performance. In unbinned images, each pixel has a certain amount of "read noise," with each pixel being read separately in an individual

"read-noise event." Sensor sensitivity in such imaging systems is partly a function of pixel size; larger pixels allows capturing more light. The drawback to unbinned images is that they take up a lot of hard drive space. However, by setting the image at 2 × 2 binning there is a compression of data, so that an array of 4 pixels get merged into one super pixel. When data such as digital photos are abundant, smaller pictures are beneficial because they are faster to process and take up less storage space. Sara explained that she preferred 2 × 2 binning, as a good trade-off between size and image quality. This time, however, she chose "bin 1" for reasons that were undisclosed.

While the scale bar and knowledge about binning may seem trivial, these settings are of epistemic importance, and play a role in propagation of representational states from the experimental system to the larger scientific community. Since image processing software depend on known pixel sizes and binning to calculate correct sizes of photographed objects, the degree of binning must be known to set parameters correctly and make the readings meaningful. The simple scale bar shows that even epistemic enhancers like photomicrographs, which extend human senses through augmenting sense modalities, require coding schemes, however minor, to be legible (Goodwin, 1994).

The final task for the day was to extract seawater from the tubes with salmon lice, and then add Karnovsky's fixative to samples, preserving them for later. This procedure was always performed under the fume hood due to the toxic formaldehyde in Karnovsky's. In this case, the intern extracted the saltwater from each tube with a micropipette and handed the tube over to Hanna, who added the fixative. Samples were then stored on ice in a Styrofoam box and brought back to the High-technology Centre for further analysis.

In the following days, all hatching incubators for the egg-strings were inspected daily down in the wet lab, and the hatching date for the eggs along with other important developments was noted in meticulous detail. Individual eggs hatch at different rates, and since early hatchers could perish before the late hatchers were fully developed into copepodites, the animals were usually collected in two batches for analysis on twelve and seventeen days after the RNAi termination. Hatched eggs were then counted around the copepodite stage, in order to learn

whether they developed through the molting phase as normal. When terminating this phase, the eggs were bathed in a mixture of 70% ethanol and saltwater. As per The Checklist's instructions, animals could at this point either be counted directly, or placed in tubes for later counting. Counting was by no means a trivial task. It was accomplished by pouring the sampled larvae into a small square container called a "counting tray," which was divided into columns. One end of this counting tray was then placed below the lens of a stereomicroscope. As the tray was gently pushed horizontally across the field of vision of the person performing the counting, juvenile parasites appearing in each column could then be enumerated by pressing a button on a mechanical laboratory counter. The number of copepodites, remaining nauplius stages, as well as unhatched egg-strings, were then inscribed into spreadsheets. These numbers made it possible to run fecundity statistics to figure out if the RNA interference had impacted biological functions, such as reproduction, by comparing the hatching rates from treated animals with those from the control group.

Concluding Remarks

Initiation and termination of RNAi experiments involve many kinds of discursive practices, such as coding, highlighting, and production of graphical representations. Together these make up a professional vision for studying gene expression in lice. The accomplishment of seeing the effects of RNAi on the louse is, following Goodwin, "lodged not in the individual mind, but instead within a community of competent practitioners" (1994: 626). To this, we can add that it is spread across situations, and artifacts. While previous chapters described the manifold branching points that led to the assembly of the experimental system, I here focused my situational analysis on representative time slices of key activities sampled from events within it. These illustrate the orchestration of material, social, cultural, and other cognitive resources that sustain the production of knowledge among molecular parasitologists.

Computer-supported phylogenetic thinking helps researchers to compare patterns of genetic sequences identified in the louse genome

with those of other organisms. This constrains the search space for salient pathways to target with RNA interference. Faced with the problem of sifting through massive quantities of data, biologists now depend on epistemic enhancers like bioinformatic software. These extend their cognitive powers and help them grasp the significance of deep evolutionary relationships between sequences, so that only the most promising candidate genes are investigated further. Computational analysis enables both an extrapolation of human senses, a conversion of information between sensory modalities, and augmentation to detect properties that human sense organs cannot access by regular means.

When exposing target sequences to RNA interference, experimentalists made use of coding schemes that systematically transformed the material world into categories and events of professional relevance. In the activities described above, we saw how a series of relatively low-level discursive practices such as browsing databases, synthesizing double-stranded RNA, picking and counting lice, injecting and repositioning them on salmon, preparing and attaching labels, punching numbers into Excel spreadsheets, taking microphotographs, making observations in the stereomicroscope, conserving samples, incubating egg-strings, monitoring fecundity and so on, facilitate higher level cognition about the function of genes. These events, by themselves mundane in appearance, show how cascades of representations are enacted, and how epistemic and pragmatic activities come together to enable the sequential propagation of representational states through the experimental system.

Action complexes for each assignment were abstractly described in The Checklist. But while this document functioned as a regulatory representation that governed several functions of the experimental system, and distributions of representations within it, The Checklist did not sufficiently specify a script with all the necessary details for accomplishing tasks critical for epistemic success. The collective had to organize themselves in a concrete situation for which there was no high-resolution plan. Hutchins points out that when we view the organization of such social events from the distributed perspective, we see that systems involving team performance sometimes remove the work of coordinating an activity away from the performing members themselves, and hands it over to structural properties of the larger activity system (Hutchins,

1995: 200). Here, we dealt with a specific type of complex coordination, the initiation and termination of RNAi, that was critical for determining the role of genes in ontogeny. While agents like Sara provided some additional coordination for certain phases of the termination procedure, much of the action was structured so that each member only needed to know what to do when certain enabling conditions occurred within the emergent ecology of the experimental system.

We also saw how pre-computations set up dependencies between elements within the cognitive ecosystem and afforded epistemic resources for RNAi trials. Information about the experiment was processed not just in the internal, biological memories of lab members, but also frequently delegated to the external environment. This illustrates two central reasons why an account of experimentation as a cultural practice must include the cognitive life of things. The cognitive properties of RNAi experiments are both removed from the properties of individual lab members through the transforming effects of tools and material environment, and through the production of emergent effects at the group level, which do not reduce to the cognitive powers of individuals.

In both the initiation and termination event, the coordinated elements included a heterogeneous collection of scientific apparatus, representational media, biological materials, human agents, and so forth. A myriad of written inscriptions provided a mutually supportive relationship between these resources in the situated arrangement of the experiment, thereby contributing to what Hacking called the "self-vindicating" structure of the laboratory sciences. As laboratory science and experimental systems mature, so are bodies of conceptual models, theories, and apparatus "mutually adjusted to each other" (Hacking, 1992: 30). All tests of theoretical and conceptual problems unfold against a material apparatus that has co-evolved along with theories, models, and different forms of data analysis, to form an interlocking, robust fit. It is the coordination between all these resources, that facilitate the kind of constraint satisfaction that made RNAi such a powerful tool for my interlocutors.

Different kinds of experimental data were coupled to each other through a myriad of written inscriptions. Coordination devices like spreadsheets, made it possible to keep track of genetically engineered lice, their offspring, and their genetic composition as they were propagated

and subjected to gene expression measurements further downstream in the system. Together, these cultural-cognitive experimental practices enacted a cascade of representations contributing to the "sifting of gold from pyrite" (Galison, 1987: 19). Sometimes, the first glimmers of the genetic pathways caused by the silencing of genes, could be glimpsed in the form of salient changes in the gross morphology and phenotypes of lice specimens that were observable by the naked eye, such as missing egg-strings and irregular body shapes. But the representational cascade did not end with observations of morphological change, of the sort visible through the eye, microscope, or from digital micrographs. These were certainly useful first approximations to answer questions about gene function, but they did not carry much epistemic weight on their own, as evidence in contexts of justification.[16] Other "filters in the space of representation" were required, to again invoke Galison's attractive metaphor. For any observation acquired via RNA interference to count as evidence for the larger scientific community, more data about the underlying molecular mechanisms involved in gene silencing was necessary. In the next chapter, we turn to how these transformations were enacted on biological tissues, further tracking how samples and their representations propagate through the pipeline. We will look at what happens to the samples placed on RNAlater, as they undergo an analytical procedure known as "quantitative PCR."

Notes

1. Efforts by the NIH to make a unified database in the 1980s exemplifies how computing was entwined with fundamental shifts in understanding gene expression over time (Stevens, 2013: 153).
2. Paralogs are genes related through a duplication event, while orthologs refer to similar sequences that are found in different species, evolving from a common ancestor. Orphan genes describe instances where a gene sequence cannot be assigned to an existing gene family due to insufficient knowledge.
3. Other relevant groupings are supergenes, neighboring genes that are inherited together due to genetic linkage and share functionality, and gene

complexes, linked genes that participate in the same biological processes, with similar but diverging functions.

4. Bayes theorem stems from a paper published posthumously in 1763 by Reverend Thomas Bayes. The foundation of Bayesian statistics, which incorporates prior beliefs into probability estimates, predates frequentist statistics by around 150 years.
5. BLAST is an abbreviation for Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).
6. See, for example, www.mrbayes.net.
7. Experimental activity involving live fish was guided by the three R's of animal testing, which encourages "replacement" or "reductions" of experimental animals where possible, and "refinement" of methods.
8. Glass needles could be purchased off-the-shelf, but the chief engineer explained that they had more success with "pulling" their own, custom-fitted to the morphological dimensions of lice targets.
9. My account is based on conversations with researchers, and reflections on my own failed effort under Ada's guidance. My cue for being in the "correct" position was that the needle no longer faced resistance from the exoskeleton when applying pressure, thus entering a softer tissue. But the tissue offers vague proprioceptive feedback, so the louse is easily skewered. Conveying this experience declaratively, beyond this, eludes my efforts. The difficulties of communicating this work propositionally, partly explains why the chief engineer, who routinely injected hundreds of salmon lice per assay, usually performed the task.
10. Fish were habituated to the tank before RNAi trials were initiated to reduce stress, indicated by their food intake and position in the water current. A precious commodity, salmonids were preferably reused for several trials, but sometimes euthanized after one single trial. Fish could also be euthanized when growing too large. Tranquillization was induced by immersing fish in a bucked for three minutes, in a combination of benzocaine and metomidate hydrochloride. Clover-oil was used to calm fish for less invasive procedures. Use of anesthetics required constant vigilance about locomotory functions and life signs. Use of salmon as laboratory animals is highly regulated, and experimenters were certified through a mandatory Laboratory Animal Science Course for Fish, introducing legal aspects of animal science, cognition, pain and nociception in fish, experimental design, and ethics. While salmon and Decapoda, like lobsters and crabs, are considered sentient under Norwegian animal welfare law, salmon lice are not.

11. Technicians eventually built an elaborate mobile installation of hoses and plastic pipes providing individual water supplies to each tank during RNAi, so that the water level could be lowered on demand to facilitate controlled infections with lice nauplii.
12. Karnovsky's has a high osmolality, a measure of the concentration of osmotically active particles in a solution and preserves cell structure with minimal alterations compared to its living state.
13. The chemical properties of RNA make it highly unstable compared to DNA.
14. Occasionally, tissues were processed directly after RNAi to biochemically capture molecules before degeneration, such as metabolites detected with enzyme-linked immunosorbent assay (ELISA), which uses antibody-markers and color to visualize substances.
15. When testing genes suspected to cause phenotypical differences in anatomical features that was not captured by the dorsal perspective, the termination team would photograph lice from other angles.
16. According to my observations, the distinction between data and evidence was not explicitly demarcated among my interlocutors. Evidence can usefully be considered a special form of contextualized data.

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