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Identification of signals describing
pairwise interaction in plankton,
bacteria and bacteriophage

A study of model, microcosm and lake

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Preface

This study concerns interaction between organisms. Such interactions are often studied through organism abundances over periods of time (time-series). Time-series reflect both internal (biological) and external (environmental) factors and the separation of these factors from time-series is a non-trivial task.

The first two papers represent an introductory stage where a new approach to time-series analysis is presented and where two-species interactions are explored with synthetic data obtained from model simulations and lake plankton data. Paper 3 reports on time-series analysis of an experimental ecological system in which interactions are studied in greater detail. Paper 4 covers experiments testing the stability of demographic trade-offs in microcosms. By doing nearly equivalent analyses within a range of “ecological realism” of the time-series, this thesis puts the suggested method through a systematic test and explores some basic assumptions in time-series analysis.

The first part of the thesis consists of a summary where this work is presented and discussed in the context of current time-series analysis. The second part contains the four papers.

The theoretical work has been carried out at the Department of Environmental Sciences at Telemark College, Norway with Professor Knut L. Seip as supervisor. The laboratory work has been carried out at the Department of Biological Sciences at Stanford University, USA with Professor Brendan Bohannon and postgraduate Christine Jessup as supervisors. My deep gratitude is extended to all of them. Also, my appreciations go to Dr. Harald Pleym for valuable assistance on Maple programming and Dr. Kim Esbensen and Dr. Sigmund Kalvenes (all at Telemark College) for guidance on statistical issues.

Høydalsmo, March 1, 2004

Gunnar Sandvik

Introduction.

“Patterns of abundance in nature are an important source of insight into the processes that determine the structure and function of communities and ecosystems”.

Orlando Sarnelle

The study of interactions among organisms is central to ecology. Ecological interactions are of many types, but are taken here to imply the detectable change in the abundance of one type of organism in response to a detectable change in the abundance of another type of organism. For simplicity, this summary refers to organism in terms of species (species interactions) although other classifications of organisms may often prove relevant. Changes in abundance (changes in population size) also result from intraspecific (within species) factors, which are not the focus of this work. To interact, the species must live in the same geographical space over relevant periods of time.

Some species interactions have been defined mathematically. Among the most important interactions are competition and prey-predator. Competition occurs when two species share limited resources and therefore the growth in one species has a negative impact on the growth in the second species. Volterra (1926) and Lotka (1924) defined competition mathematically. Prey-predator is the species interaction occurring when one species is the predominant prey of another species and the predator depends on its prey for reproductive success. The reduction in the prey population is followed by a reduction in the predator population giving the prey population a new opportunity to increase and thus leading to a cyclic pattern of growth and decline in the two populations. Volterra (1926) and Lotka (1924) independently formulated the prey-predator interaction mathematically. Mutualism means that growth of the population in one species has a positive impact on the growth of the population in another species and vice versa. Mutualism is well documented (e.g., pollinator and flower, algae and fungi in lichens) in various ecosystems (May 1976, Boucher et al. 1982 and Wright 1989), but is less developed mathematically because, if unconstrained, it implies autocatalysis or unlimited and accelerated growth (Roughgarden 1998). Facilitation is the re-allocation of resources by one species causing population growth in another species (Thayer 1979, Naiman et al. 1988, Jones et. al. 1994 and Lawton & Jones 1995). Examples are beavers diverting water for the benefit

of other species or woodpeckers producing nesting sites for other species. Mathematical formulations for each of the four interactions are given in paper 1.

This thesis focuses mainly on prey-predator and competition, which are by far the most studied of the species interactions.

What is the significance of knowing species interactions?

The motivation for studying species interactions has two main aspects. Firstly, an individual species live in dynamic relationships with other species. Thus, to further increase the knowledge about individual species as well as the ecological systems they live in, science must look at interactions. Secondly, with the increased understanding of ecological principles, it has become apparent that efforts to protect single- or multiple species, must rely on a thorough understanding of species interactions.

If species interactions and their strengths could be identified with reasonable certainty from time-series, it would be useful to both science and management.

Inferring process from pattern

The hypothesis of this thesis is that "unique signatures" for different species interactions are imbedded in time-series and that these "signatures" are largely invariable across ecologic systems. Such tacit "signatures" are termed signals in this thesis and a signal and its strength are defined mathematically (papers 2 and 3).

There are fundamental differences between the interactions of prey-predator and competition and these differences are reflected in phase portraits (two dimensional plot with counts or biomasses of species 1 on the x-axis and species 2 on the y-axis, Fig 1 below). Theory predicts that in a phase portrait the biomasses of a stable prey-predator pair will show sustained oscillations (limit cycles) with certain amplitude and period as well as a counterclockwise rotational direction if prey is depicted as the x-axis species and predator is depicted as the y-axis species (Lotka 1924, Volterra 1926 and Case 2000). On the other hand, theory of (resource) competition (Lotka 1924 and Volterra 1926) predicts the extinction of

one species in most situations. Experimental results have supported this prediction (Gause 1934 and Tilman 1981).

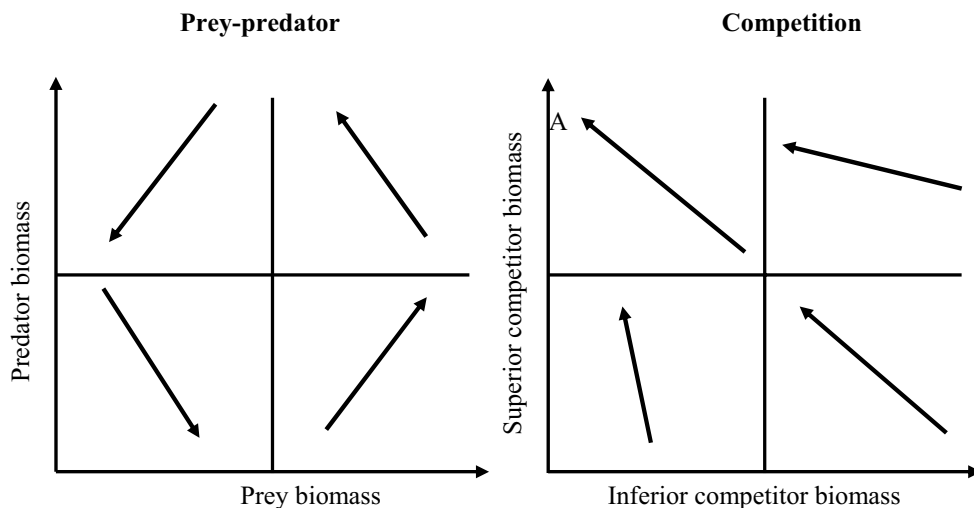


Figure 1. Schematic phase portraits illustrating typical theoretical predictions for the prey-predator and competition interactions. Different initial conditions in terms of combinations of high and low biomasses give different results. In the prey-predator interaction a circular motion is predicted, while in competition all arrows are predicted to wind up in point A (i.e., extinction of the inferior competitor). **Note:** with certain combinations of carrying capacity (i.e., population size where population growth is zero) and α values (i.e., degree to which species 1 inhibits the growth of species 2) the species may coexist or the superior competitor may even become extinct.

Based on theory the prey-predator interaction may therefore be easier to identify than competition since a strong prey-predator interaction is expected to produce recurrent dynamics while a strong competitive interaction is not (pronounced continual cyclic pattern vs. transient pattern).

However, the observation that species obviously exist over long time-scales implies that competition must be viewed in the context of other factors than those included in the Lotka-Volterra model. One important factor is the ecological niche (i.e., a species has a number of

requirements in terms of resources and living conditions). Appreciating that extinction is not the rule in real ecological systems, Hutchinson (1959) developed the concept of “limiting similarity” (i.e., how different two species have to be in terms of niche requirements to coexist). MacArthur & Levins (1967) provide a theoretical framework for this principle using a three-species Lotka-Volterra model.

In ecological systems, resources and living conditions often vary continuously. For a pair of similar species, we may expect alternating periods of strong, intermediate, and weak or no competition (McCann et al. 1998 and Matveev 1995) as well as variation in the relative roles of different interactions (Bohannan and Lenski 2000b). Also, in most natural systems interactions between pairs of species do not form closed “sub-systems” (predators alternate between different prey, and prey is killed by various predators).

One of the best-studied ecological time-series involves lynx and snowshoe hare (Elton & Nicholson 1942). Both the ecological roles of these species (the carnivore lynx and the herbivore hare) as well as their overlapping geographical distributions, strongly suggest that prey-predator dynamics are reflected in the time-series, but this finding is debated (Gilpin 1973 and Boutin et al. 1995). This does not imply that lynx does not prey on hare; the discussion is rather about our models of prey-predator dynamics and how well they are supported by the data.

Exploitative competition (i.e., individuals of one species suffer a reduction in growth rate from the growth of a second species due to their shared use of limiting resources) has been observed in natural systems when an external superior competitor is introduced to the habitat (i.e., living area that satisfy the niche requirements of a species) of an inferior competitor. The spread of (initially five) introduced muskrats from Prague in the Czech republic (Elton 1958) and the spread of Argentine ants around San Diego, California (Erickson 1971) provide illustrative examples. These competitive exclusions (spread of invasive species) would be detectable from relevant time-series, but what about the more common situation in most ecological systems where competing species live alongside for long periods of time?

Population cycles in voles and lemmings have long been debated as being either predator controlled (“top down”) or resource controlled (“bottom up”) (i.e., whether the time-series represent prey-predation or not), (Turchin et al. 2000 and Stenseth 1999). Similarly, the

relative contributions to time-series by “top down” and “bottom up” forces are discussed in lake studies (McQueen et al. 1986 and Matveev 1995) and laboratory systems (Bohannan & Lenski 1997 and 2000a). Identification of prey-predator and competition from time-series is an area of active research in ecology.

Additional challenges associated with identifying interspecific interactions from ecological time-series come from external factors (noise) such as environmental stochasticity (randomness) and seasonal forces (predictable fluctuations) which influence population dynamics (Chesson & Huntly 1997). The question addressed in this thesis, is how species interactions (prey-predator and competition) can be identified from relevant pairs of time-series without making assumptions about the mechanism of the interactions. The thesis tests the suggested methodological approach with three fundamentally different types of time-series data (i.e., model, experiment and lake) to evaluate the relevance and robustness of the approach.

Methodological approaches to time-series analysis in ecology

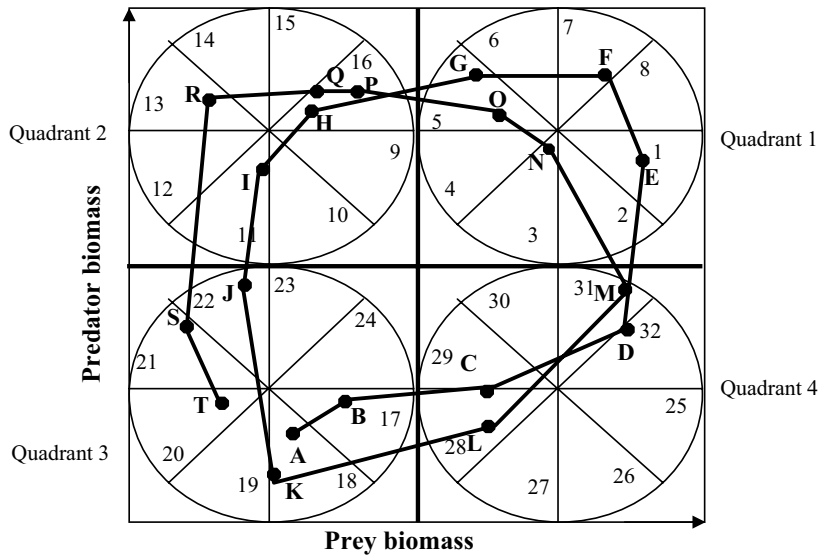
Several methodological approaches have been proposed for the study of species interactions, but a major distinction is made between statistical analysis of time-series and mechanistic modeling. The statistical analysis approach studies properties of time-series assuming that they reflect the species interactions and that irrelevant information can be “filtered out”. The mechanistic modeling approach makes assumptions about crucial process mechanisms and aims at predicting relevant future time-series (Kretzschmar et al. 1993, Scheffer et al. 1997 and Murdoch et al. 1998). Mechanistic models are limited by being strongly dependent on knowledge about biological model parameters and by becoming mathematically complex when the number of species increases (Roughgarden 1998, Kristoffersen 2001 and Moe 2001). Statistical approaches use well-developed statistical tools. These approaches make fewer assumptions about the ecological processes, but may be criticized for being biologically naive, because they treat time-series only as “strings of numbers” (Kendall et al. 1999). Applying the “goodness of fit” criterion to only one or a few time-series also involves the additional risk of these “realizations” not being representative of the ecological process under study (Jost & Arditi 2000).

The most common approach to present time-series analysis in ecology is various autoregressive models. In such statistical models the biomass of a species is seen as a delayed function of the same species' biomass at earlier time points in a time-series (Matveev 1995, Stenseth et al. 1997 and Berryman & Turchin 2001). Time-series have also been analyzed as regressions between biomass measures of species with different ecological roles (McQueen et al. 1986, Sarnelle 1994, Mazumder & Havens 1998 and Matveev 2003). More pattern-oriented approaches are statistical analyses of peak shapes and frequencies and the use of Kendall and Stuart's test (Kendall & Stuart 1966) for detection of regularities in time-series (McCauley & Murdoch 1987 and Turchin et al. 2000). Other recent approaches combine statistical modeling with mechanism-based models (Kendall et al. 1999, Jost & Arditi 2000, 2001, Kristoffersen 2001 and Turchin et al. 2003).

The AFM approach and how it relates to other approaches

The new methodological approach suggested and tested in this thesis is graphical and statistical (the angle frequency method, AFM). AFM is a further development of the traditional study of phase portraits in ecology combined with principal component analysis (PCA) (Seip 1997 and Seip & Pleym 2000). The rationale for this approach is the assumption that various types of ecological interactions will show unique signals in phase portraits.

In a phase portrait, synoptic pairs of observations of the density (abundance) of two species are merged into one ("two dimensional") point of a trajectory. Each phase portrait is divided into four quadrants (see fig. 1 and fig. 2.). Quadrant 1 (upper right) represents all sections of the trajectory in which both species show abundances above their average abundance. Quadrant 3 (lower left) represents the opposite situation (both species below their average abundances). Quadrants 2 and 4 represent sections of the trajectory where one species is above and one species is below their average abundances. The AFM approach increases the "analytic resolution" of traditional analysis of phase portraits by focusing on species trajectories in four quadrants (for the present, four, it could be more). Within each of the four quadrants the AFM defines 21 sectors (21 by choice) of a full circle (see fig. 2 below).



Signal	4	5	6	8	11	13	18	24	30	31	32
Frequency	1	3	1	1	2	1	2	3	1	2	1
Trajectory	GH	FG,NO,OP	EF	F	HI,RS	PQ	JK,ST	AB,BC,KL	MN	DE,LM	CD

Figure 2. Phase portrait showing an example prey-predator time-series (2 cycles). For illustration purposes, each quadrant has only 8 sectors of a circle (the real AFM has 21 sectors). Letters show time points (pairs of successive biomass values). The table shows angles of vectors (trajectories connecting pairs of successive time points) and the frequency of these occurrences for this particular time-series. Only the non-zero sectors (signals) are included in the table.

The first step in the analysis of a phase portrait is the identification of relevant sectors (termed “angle brackets” in paper 1 and signals in papers 2 and 3) and the frequencies by which these signals occur in a given pair of time-series (i.e., phase portrait). This first step may be viewed as converting individual phase portraits into signal frequency tables (fig.2). This is a computerized operation (Maple V, Release 5, Waterloo Maple, Inc., 1998).

The second step is a comparison of phase portraits by their signal frequencies (table in fig. 2). Such comparisons are done by principal component analysis, PCA (Unscrambler, version 6.11 b, CAMO ASA, 1997). PCA is concerned with reducing the total spread in the values of

a variable (variance) and transforming the linear relationship between variables (covariance) in a data set into a few *linear* combinations of the original variables (Johnson & Wichern 1992).

PCA is primarily used for data reduction (i.e., reduce total data variability into a few principal components containing almost the same information) and interpretation (i.e., detect “hidden” information from complex data sets) (Johnson & Wichern 1992 and Esbensen 2000). The first principal component PC1 is the maximum variance direction in a data “swarm”. If, for example, three original variables are strongly correlated, the effective dimension may not be three, but closer to one and therefore PC1 may be a good approximation. Similar correlation structures may be identified for successively lower directions of variance (i.e., PC2, PC3, etc.), but the total number of PC’s is nearly always much lower than the number of variables in the original data (Esbensen 2000). The principal components are uncorrelated (orthogonal).

The PCA input data is a matrix consisting of objects (rows) and variables (columns). In our application the variables are the 84 possible signals and the objects are individual phase portraits. Each entry (cell) of the data matrix is the frequency of a signal for a particular phase portrait.

The next step of the AFM analysis is a comparison of two (tentative) groups of phase portraits (e.g. groups expected by the analyst to represent either prey-predator or competition interactions).

The relevant PCA outputs are the scores and the loadings (defined below). The scores quantify the relationship between objects (phase portraits) along a principal component. The first principal component is the direction of highest variance in the data, thus if the largest difference between phase portraits is due to interaction (i.e., prey-predator or competition) this would be reflected in the scores.

In order to estimate how well interactions are separated from each other by their signal frequencies (i.e., how well the tentative grouping is supported), we define percentage discrimination along a principal component as (1).

$$(1) \quad D\% = \frac{D * 100}{R_n}$$

$D\%$ is a measure of the distance, D (equation 2) between the average scores in each of two clusters of scores expressed in percent of total range, R_n (equation 3) of scores along the relevant PC axis. A $D\%$ value of ~ 100 implies that scores are separated as two distinct groups at opposite sides along the PC axis. A $D\%$ value of ~ 0 represents no grouping in the scores. $D\%$ values in the range 10-15 are found to be visually detectable by inspection of score plots.

$$(2) \quad D = |g_1 - g_2|$$

D is the absolute center - center distance; g_1 is the average score of group 1 and g_2 the average score for group 2 along a given PC-axis.

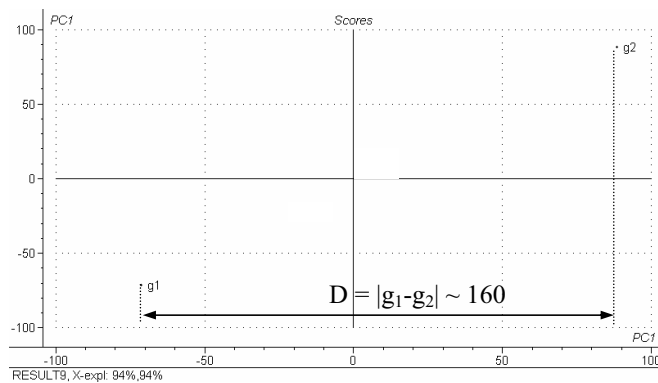


Figure 3. Example score plot with D value calculated along PC1, for a pair of group averages. (Exact figure $D_{d1-d2} = |-71.435 - 88.279| = 159.714$).

$$(3) \quad R_n = g_{\max} - g_{\min}$$

R_n is the range of scores. Where g_{\min} is the lowest score and g_{\max} is the highest score (irrespective of groupings among scores) along a given PC-axis.

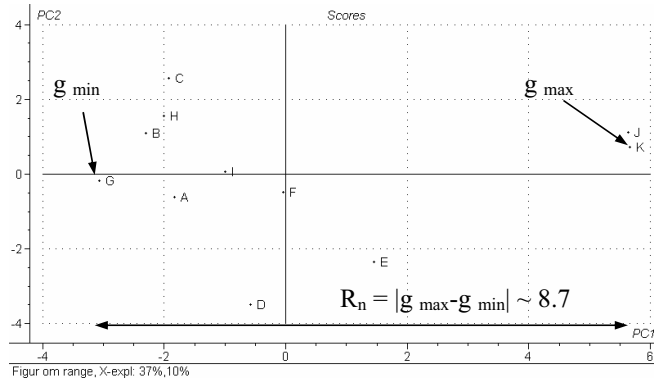


Figure 4. Example score plot with R_n value calculated along PC1. (Exact figures $R_n = 5.655 - (-3.070) = 8.725$). A,B,C,.....K scores.

For instance, high discrimination along PC1 would be the result if phase portraits assumed to represent competition largely show negative score values while phase portraits assumed to represent prey-predator interaction largely show positive score values. If discrimination is good (i.e., the PCA analysis supports our tentative grouping of phase portraits) we want to know, which signals in a given analysis, are associated with prey-predator and competition respectively?

PCA loadings relate the variables to the score. The variables represent each of the 84 signals.

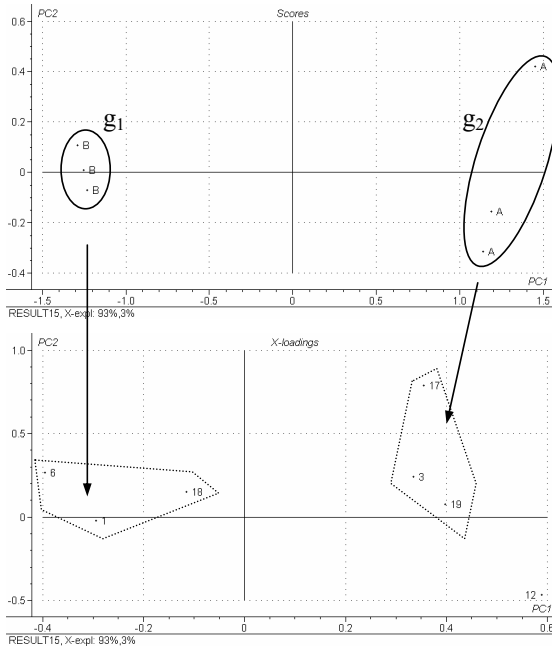


Figure 5. Example score plot and loading plot. The score cluster g_1 is associated with variables (signals) 1, 6 and 18. The score cluster g_2 is associated with variables 3,17 and 19.

Based on loadings (lower panel in fig 5) associated with g_1 and g_2 scores (upper panel in fig 5), we define the signal strength S_s . High S_s values point to signals that are good candidates for identifying a given interaction type. For a signal to have a high S_s value for competition, the signal loading must occur frequently in association with score clusters assumed to represent competition. The mathematical definition and further explanation of this parameter is given in papers 2 and 3.

Unlike autoregressive models, the AFM approach does not make the assumption that the biomass at time point t can be predicted from biomasses at earlier time points ($N_t = F(N_{t-1}, N_{t-2}, \dots, N_{t-d})$). A critical factor in such analyses is the identification of relevant time lags representing the biological feedback process (how an earlier time point influences a later time point) or the number of previous time steps to include in the analyses (Berryman & Turchin 2001).

Regression models suffer from the prerequisite that the response variables should be largely uncorrelated, which they most often are not in time-series from the same ecological system (Kristoffersen 2001). The linear autoregressive models assume linear relationships between response and predictor variables (e.g., a linear relationship between biomasses at time points t and $t-1$), both assumptions are often violated in natural time-series (Berryman & Turchin 2001 and Kristoffersen 2001).

Some studies assume that interaction between two species can be identified from the time-series of only one species (e.g., Turchin et al. 2000 and Berryman 2001). By simultaneously studying two time-series (one phase portrait) we avoid assumptions about how pairwise interactions are reflected in one-species time-series.

The strength of the AFM approach is that it does not make any *a priori* assumptions about dynamic properties of the time-series; clustering will occur if there are inherent similarities between species pairs that partake in similar interactions.

Study systems

A major goal of this study is to test whether the same signals of prey-predator and competition can be identified both in model simulations, experimental microcosm systems and in observed lake data. It would support the hypothesis if species interactions were identified by the same signals in these different study systems.

The **mathematical models** used here representing the species interactions, prey-predator, competition, mutualism and facilitation are all relatively simple (“Lotka-Volterra type”), including inter- and intraspecific density dependence (see Seip & Pleym 2000 and paper 1). Simulations of these basic models are modulated using a simple approximation to seasonal cycles by using a Gauss function producing regular variability in conditions for growth over one simulated year (i.e., seasonal forcing, details in paper 1). Such seasonal forcing is a crude proxy for all the environmental parameters that limits population growth in a cyclic manner in real seasonal ecosystems (e.g., temperature, day length, irradiation, wind speed, etc).

The mathematical models serves two purposes in this thesis. Firstly, they render a systematic study of the influence of seasonal forces on the structural stability of modeled species interactions (reported in paper 1). Secondly, by imposing seasonality and by varying key parameters (testing sensitivity), model simulations produce time-series relevant for a comparison with time series from seasonal lake and microcosm time-series (reported in the discussion of this thesis).

Competitors are simulated as being equal in terms of their resource requirements, but different in their response to seasonal forces. Predators are simulated with a standard type II functional response (i.e., if the number of potential prey rises, the number of actual prey also rises at a decreasing rate toward a maximum).

Mathematical models in ecology are always approximations (they do not establish “laws” of ecology), but they may be highly useful tools in the formulation and discussion of ecological theory (Roughgarden 1998).

The **microcosm studies** use experimental glucose limited (Tilman 1977, 1981) chemostat cultures (Chao et al. 1977, Lenski 1988b and Bohannan & Lenski 1997). These systems are suitable for producing time-series because they are continuous (resources are supplied and excrements and dead organisms are removed at constant rates). In these highly controllable systems several experiments were carried out (papers 3 and 4). The organisms used to represent prey and competitors in this study are bacteria, which are primitive prokaryote organisms. Bacteria are mostly unicellular organisms with a metabolic machinery fixing energy from various chemical reactions (a few have photosynthesis) (van den Hoek et al. 1995). The bacteria we use are strains produced by experimentally induced evolution from ancestral *Escherichia coli* B (papers 3 and 4).

The organisms used to represent predators are bacteriophage (phage). Phages consist of genetic material (DNA) or (RNA) encapsulated in a protein coating and are primitive organisms. These minute parasites invade plant-, animal- and bacteria cells and use their metabolic machinery to complete their own life cycle (Wallace et al. 1986). The viruses we use are T-series bacteriophage (especially T4, T2 and T7). T-series bacteriophage adsorbs to the surface of *E. coli* cells where they insert their DNA. Phage DNA “reprograms” the

bacteria cell to start producing phage progeny (Lenski 1988b, Goldberg et al. 1994 and Bohannan & Lenski 2000b).

A trade-off between competitive ability in the bacteria and resistance to the phage is maintained to stabilize the population dynamics in the microcosm studies (Lenski 1988a, 1988b and Simms 1992). This trade-off is a result of most phage-resistant strains achieving their resistance through the loss or modification of the receptor molecule to which the phage initially binds. These modifications often simultaneously reduce the cell's competitiveness because the receptor molecules are also involved in bacterial metabolism such as the uptake of nutrients (Lenski 1988a).

Although viruses are strictly parasitic, their interaction with bacteria is viewed as a good approximation to a prey-predator interaction (Levin et al. 1977, Chao et al. 1977, Lenski 1988b and Carlson & Miller 1994).

The lake study (paper 2) analyses plankton time-series from three temperate **seasonal lakes** (Lakes Mjøsa and Farris in Norway and Lake Washington near Seattle in the USA). Plankton is subdivided into phytoplankton (mainly algae and cyanobacteria) and the zooplankton (animals of plankton size), mainly Protozoa, Rotatoria, Cladocera and Copepoda) (Cole 1983). Phytoplankton organisms mainly drift in the water because they are too small or too weak to have a significant motion of their own. Zooplankton can move somewhat, at least vertically. In general, the phytoplankton produces biomass that is consumed by the zooplankton.

Plankton in temperate seasonal lakes has alternating low growth rates in the autumn/winter and high growth rates in the spring/summer. Seasonal variation in the availability of limiting nutrients is a strong community-shaping factor (Tilman et al. 1982). Each season in such lakes consists of a succession where populations of several plankton species increase and decline in a sequential order. The order may vary substantially among the years (Makulla & Sommer 1993). However, the empirically based PEG model identifies “typical” stages of the seasonal succession, in which larger groups of plankton play important roles (e.g., the “spring bloom” in phytoplankton occurring in early spring when resources are ample, but

before resource scarcity (due to competition) and grazing (from zooplankton) reduce total phytoplankton numbers) (Sommer et al. 1986).

Because most lakes are complex natural systems (i.e., high numbers of species occurring simultaneously) there is no direct way of controlling whether a pair of time-series actually represents the interaction we would assume on the basis of theory and experiments in the laboratory.

List of Papers.

Paper 1: Gunnar Sandvik, Knut L. Seip & Harald Pleym. 2002. *An anatomy of interactions among species in a seasonal world*. *Oikos* 99: 260-271.

Paper 2: Gunnar Sandvik, Knut L. Seip & Harald Pleym. 2003. *Extracting signals of predation and competition from paired plankton time-series*. *Archive für Hydrobiologie* 154(4): 455-471.

Paper 3: Gunnar Sandvik, Christine Jessup, Knut L. Seip & Brendan Bohannon. 2004. *Using the angle frequency method to detect signals of competition and predation in experimental time series*. *Ecology Letters* 7(8): 640-652.

Paper 4: Brendan J. M. Bohannon, Ben Kerr, Christine Jessup, Jennifer Hughes, & Gunnar Sandvik. 2002. *Trade-offs and coexistence in microbial microcosms*. *Antonie van Leeuwenhoek* 81: 107–115.

Summary of the papers.

Paper 1: An anatomy of interactions among species in a seasonal world.

In this paper, synthetic time-series were generated from standard models representing four species interactions (prey-predator, competition, mutualism and facilitation). To study the influence of seasonal forces on these interactions, the growth rates of the models are modified by a simple approximation to a seasonal cycle. Measured as system variability (i.e., variance of AFM angle frequencies) prey-predator dynamics is strongly affected by seasonality, while the dynamics of the three other interactions are moderately affected. When difference in growth optima is introduced (i.e., niche difference), competition intensifies, while mutualism remains unchanged. Following a 20% variation in key parameters, interactions could still be separated and characteristic signals identified by PCA.

Paper 2: Extracting signals of predation and competition from paired plankton time-series.

Plankton time-series from three seasonal lakes were analysed by AFM. The aim of the paper is to search for signals identifying species interactions (competition and prey-predator) across lakes. The paper also explores various methods of data aggregation aiming at signal amplification. Prey-predator and competition (phase portraits) are separated, but high levels of noise in the data, give relatively weak signals. Prey-predator signals identify the predator collapse at low prey density and a counter-clockwise rotation (i.e., supporting the Lotka-Volterra model). Signals assumed to represent competition are difficult to interpret within the framework of classical competition theory.

Paper 3: Using the angle frequency method to detect signals of competition and predation in experimental time series.

Experimental time-series with bacteria and bacteriophage from continuous chemostat cultures were analysed by AFM. The aim of the paper is to identify signals of prey-predator and competition interactions in time-series from communities, where both these interspecific

interactions occur simultaneously, as is common in real ecosystems. A secondary aim is to evaluate the usefulness of the AFM approach in studying other aspects of time-series, (e.g., detecting effects of invasion and resource enrichment). Prey-predator interactions gave several strong signals. The strongest signal was the predator collapse at low prey density. By using competitors of known fitness (competitive strengths), we also identify relatively strong signals of competition. However, competition signals are sometimes difficult to distinguish from indirect effects of the simultaneous prey-predator interaction. Effects of invasion and resource enrichment were also detected.

Paper 4: Trade-offs and coexistence in microbial microcosms

In this paper we use microcosms with the bacterium *E.coli* and T-type bacteriophage to study and discuss various aspects of ecological trade-offs. In this experimental system, the trade-off arises when the bacterial resistance to predation (from the phage) comes with a cost in competitive fitness of the bacteria (high cost implies large trade-off). Such trade-offs tend to stabilize community dynamics and secure long-term coexistence of competitors. The experiments show that the cost of resistance depends on environmental factors such as resource levels, type of limiting resource and environmental stability (batch culture vs. chemostat culture). Thus, the magnitude and nature of such trade-offs may serve as predictors of how ecological communities respond to various environmental changes.

Discussion

In paper 1 we observed interference from seasonal rhythm with the biological processes. The finding, that prey-predation is strongly influenced by periodic forcing (i.e., seasonality) supports other findings (Hastings et al. 1993 and Tømte et al. 1998). When periodically forced, simple prey-predator models may show a range of behaviours and therefore periodic forcing should be an essential ingredient in the study of prey-predator models, if we want to use them to understand this interspecific interaction in real world seasonal systems (Scheffer et al. 1997).

Synthetic time-series (prey-predator, competition, mutualism and facilitation modulated with periodic forcing and simulated over a range of parameter selections) could all be distinguished by AFM.

The search for signals in time-series has led to another interesting result. Comparisons of papers 1 to 3 show that prey-predator and competition are separable in all three types of time-series. As expected the methodological trade-off between realism and control (i.e., in ecological studies high control usually means low realism while high realism correspondingly implies lower control) is reflected in the results. The grand average discrimination between prey-predator and competition is 88 in the model (paper 1), 42 in the experiment (paper 3) and 26 in the lake study (paper 2) (a discrimination of 100 means full separation and a discrimination of 0 means no separation or randomness).

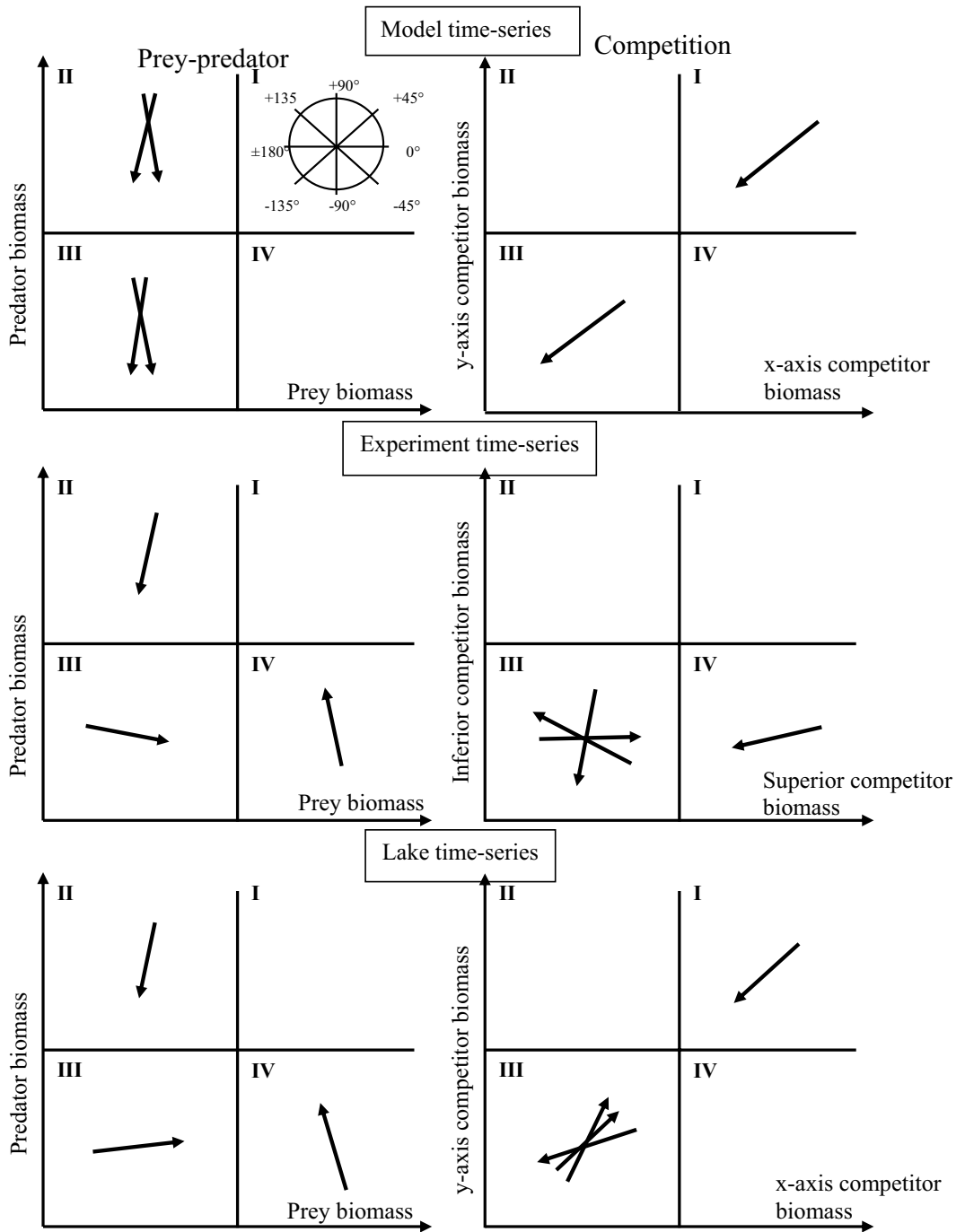


Figure 6 Strong signals identified from time-series in papers 1-3. Rows give type of time-series (model, experiment and lake). Phase portraits in column 1 show prey-predator signals, phase portraits in column 2 show competition signals. The circle gives the angles of arrows.

Despite the relatively large difference in overall discrimination among the systems discussed in the individual papers, the same signals were largely identified as strong for the prey-predator interaction. Fig 6 shows a considerable similarity in prey-predation signals across types of time-series. All signals are in agreement with the counter-clockwise rotation of trajectories (Lotka 1924 and Volterra 1926). Furthermore, one signal (downward facing arrow in quadrant 2, i.e., the predator collapse at low prey density) is a strong identifier of the prey-predator interaction in all three types of time-series (see papers 1 to 3). Signals from lake and experimental time-series fit better with model assumptions (i.e., Lotka Volterra) than the analysed model time-series, suggesting that the combination of seasonal forcing and parameter modifications have masked relatively much of the underlying dynamics.

For competition, the results are not equally comparable because competitors are more heterogeneous and also defined somewhat differently in the papers. In the model study the two competitors are identical except from one being the “early starter” (see paper 1). In the microcosm study the competitors are similar, but differ in fitness and sensitivity to predation (see paper 3). In the lake study the competitors are different species of phytoplankton included in the analysis, because they occur several months each growth season and in each year of the time-series.

No single signal identifies competition in all types of time-series. However, a signal that would be expected on the basis of theory, is found to be strong in both model and lake time-series. This signal (downward facing arrow in quadrant 1) shows that when both competitors occur in high densities, they decline at approximately equal rates. This is what would be expected when competitors are equal or similar (competitors have equal competition coefficients in the model time-series) and when both populations are high.

These results indicate that competition signals are more difficult to identify from time-series than signals of prey-predator. A probable reason is the oscillatory nature of prey-predator interactions. For competition no such cyclic or repetitive pattern is predicted. Resource competition for one or two limiting resources between two species (Tilman 1981), if not balanced by external forces like prey-predator (Chase et al. 2002) or environmental factors (Chesson & Huntly 1997), will normally produce one winner and one loser. In the time-series

analyzed, the prey-predator interaction (papers 2 and 3) and seasonality (papers 1 and 2) modulated competition and probably (masked) its signals in the time-series.

In paper 3, signals of the prey-predator interaction are strengthened by enrichment. This supports classical theory predicting that enrichment can destabilize a prey-predator interaction, leading to increased amplitude and period of population oscillations (Rosenzweig 1971) and experimental results (Bohannan & Lenski 1997, 1999 and 2000a).

Small differences in bacterial fitness are identified from experimental time-series in paper 3, but similar competitors gave weaker signals of competition than more dissimilar competitors. This supports classical theory and experiments, which predict (Lotka 1924 and Volterra 1926) and demonstrate (Gause 1934 and Tilman 1981) more drastic effects on populations, when competitors are different (i.e., extinctions or considerable depression of the inferior competitor in systems with one limiting resource). On the other hand, the result contradicts the limiting similarity principle (Hutchinson 1959), which assumes that the strongest effects of competition (i.e., extinction or severe depression of the inferior competitor in systems with one limiting resource) will occur when competitors are similar. A recent review article notes that there is considerable confusion as to how to define strength or intensity of competition in the scientific literature (Chase et al. 2002).

Conclusion

This thesis demonstrated that prey-predator and competition interactions can be discriminated in the three different types of time-series (model, experiment and lake) and that the prey-predator interaction is identified by largely the same signals in all types of time-series. These signals correspond well with theoretical predictions (classical prey-predator dynamics). The thesis failed to identify a similar type of signals for the competition interaction. The signals that were identified could plausibly be explained as combined effects of competition and seasonal forces (paper 2) and combined effects of competition and predation (paper 3). The thesis also demonstrated that the magnitude of the trade off between relative fitness and

susceptibility to predation in *E.coli* bacteria is dependent on environmental factors including the type and concentration of limiting nutrient and on environmental stability.

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