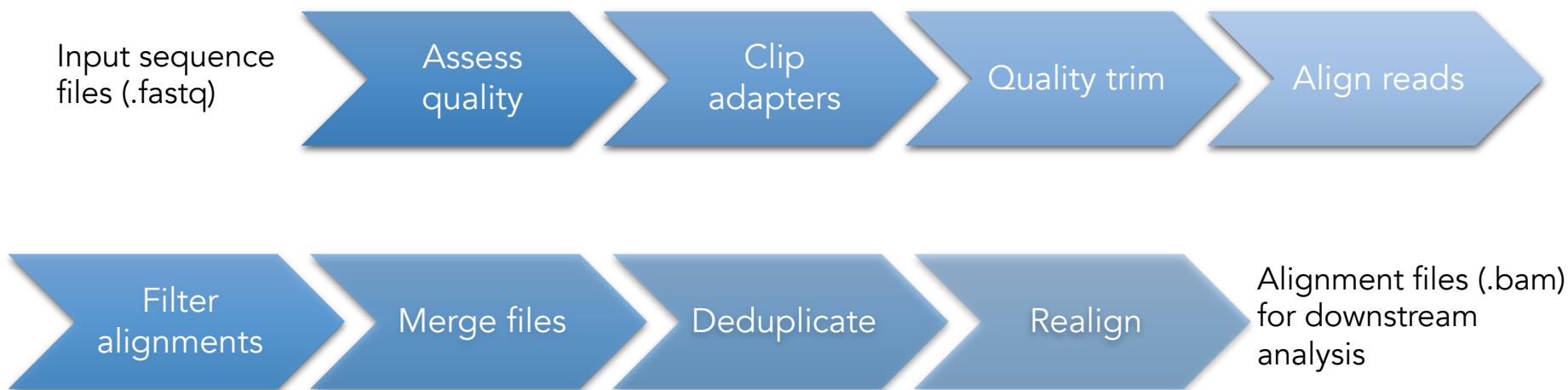


# From fastq to bam

# Bioinformatic pipeline



Assess quality

Clip adapters

Quality trim

Align reads

# FastQC

Nice tool for diagnosing problems with your data



Assess quality

Clip adapters

Quality trim

Align reads

# FastQC

Nice tool for diagnosing problems with your data

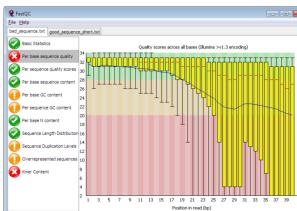
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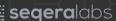
**FastQC**

Function	A quality control tool for high throughput sequence data.
Language	Java
Requirements	A suitable Java Runtime Environment The <a href="#">FastQC BAM/SAM Libraries</a> (included in download)
Code Maturity	Stable. Mature code, but feedback is appreciated.
Code Released	Yes, under <a href="#">GPL v3 or later</a> .
Initial Contact	Simon Andrews

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Latest release: v1.16  
9 days ago  
Citations: 3.9k

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**Aggregate results from bioinformatics analyses across many samples into a single report**

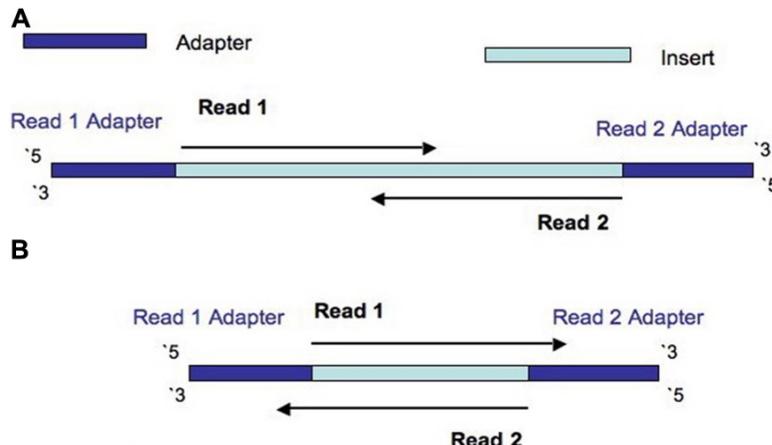
Assess quality

Clip adapters

Quality trim

Align reads

## Read adapter read-through sequence



- If the library insert is shorter than the read length, the end of the read will be adapter sequence
- Adapter sequence can interfere with mapping and variant calling

Assess quality

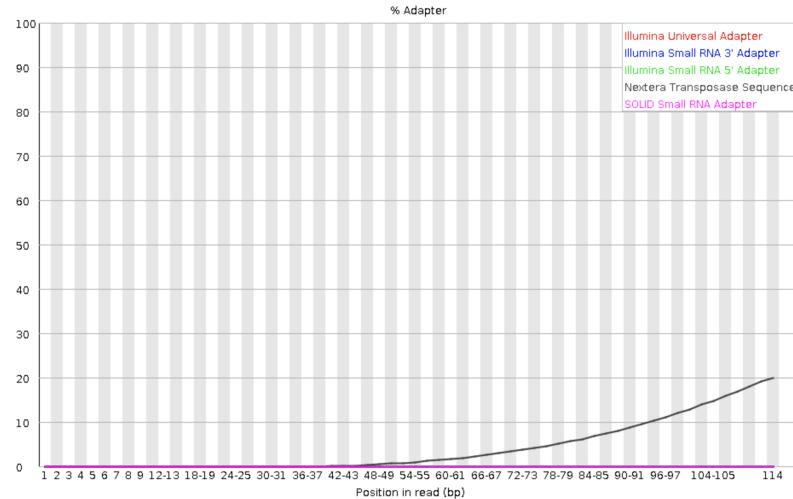
Clip adapters

Quality trim

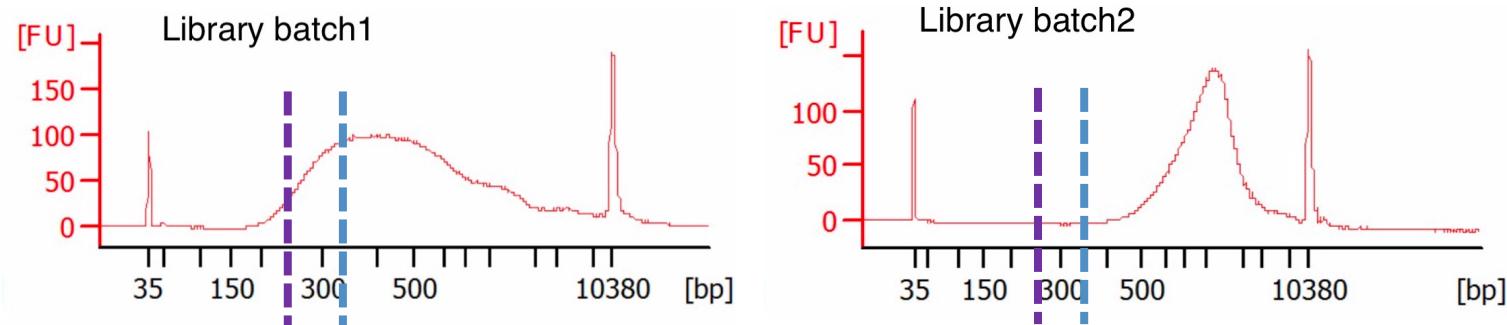
Align reads

## Read adapter read-through sequence

### ✖ Adapter Content



# Two examples of our library pools



The length of Nextera adapters is 138 bp and libraries were sequenced with 2\*125bp reads

- Minimum fragment length to avoid overlap 388bp
- Minimum fragment length to avoid adapter read-through 250bp



## Base call quality scores

Measure the probability that a base is called incorrectly

$$Q = -10\log_{10}(e)$$

where  $e$  is the estimated probability of the base call being wrong

- **Higher Q scores** indicate a smaller probability of error
- **Lower Q scores** may lead to false variant calls

Quality score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%

Assess quality

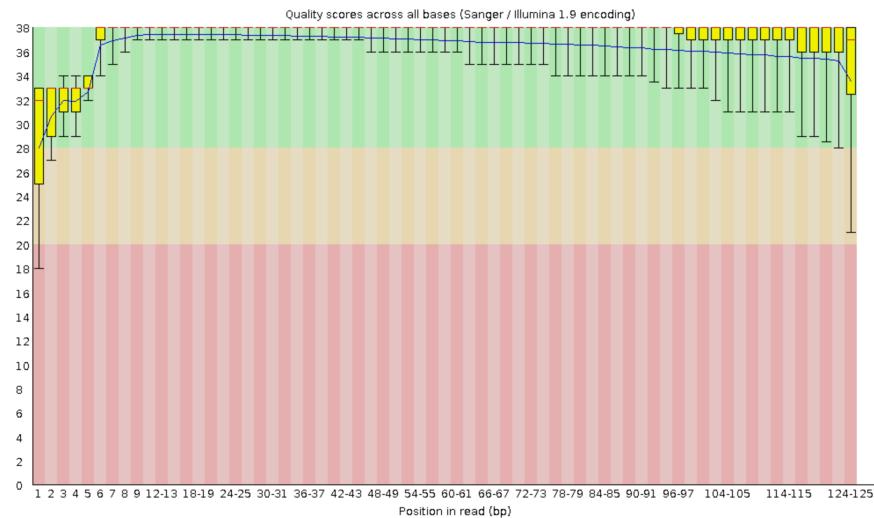
Clip adapters

Quality trim

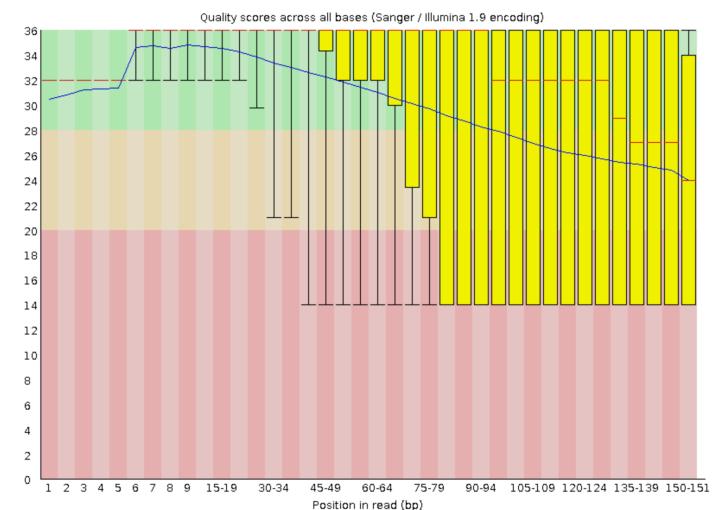
Align reads

Sequence quality can vary dramatically between sequencing runs

Per base sequence quality



Per base sequence quality





## Quality trimming is optional

- Probabilistic downstream analysis frameworks take base quality scores into account
  - Retaining base calls with a 99% probably of being correct may be more valuable than discarding it (zero information)
- However, base call quality scores may be mis-calibrated (i.e. not reflecting the true probability of being correct)
- Low quality data can add noise to analysis

Assess quality

Clip adapters

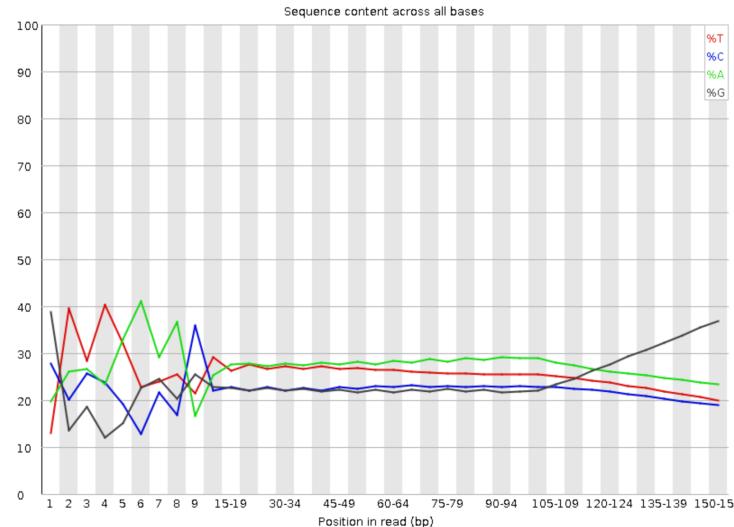
Quality trim

Align reads

Other potential quality issue: poly-G tails in two-channel sequencers



#### Per base sequence content



Can remove with dedicated poly-G trimming software, but we have seen better results from simple sliding window quality score trimming

Lou, R. N., & Therkildsen, N. O. (2022). Batch effects in population genomic studies with low-coverage whole genome sequencing data: Causes, detection and mitigation. *Molecular Ecology Resources*, 22(5), 1678–1692.

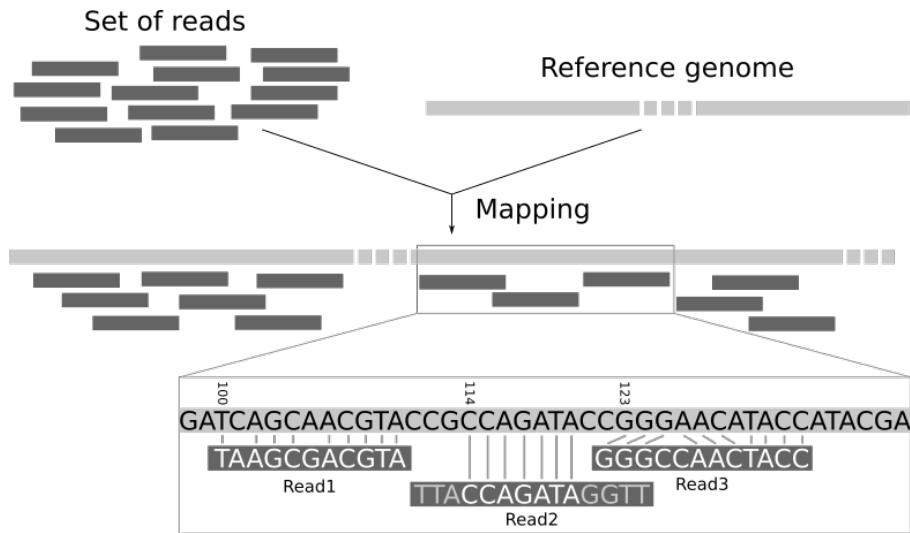
Assess quality

Clip adapters

Quality trim

Align reads

## Mapping to a reference sequence





## Filter alignments

- Not always possible to map reads to their point of origin in the genome
  - E.g. duplicated and repetitive sequence
- Each alignment given a mapping quality (MapQ)

$$Q = -10\log_{10}(p),$$

where p is an estimate of the probability that the alignment does not correspond to the read's true point of origin

- Mapping quality related to uniqueness
- Reads that map in multiple places can bias analysis

MapQ	Probability that read truly originated from different place
10	1 in 10
20	1 in 100
30	1 in 1000



## Filter alignments

- Not always possible to map reads to their point of origin in the genome
  - E.g. duplicated and repetitive sequence
- Each alignment given a mapping quality (MapQ)

$$Q = -10\log_{10}(p),$$

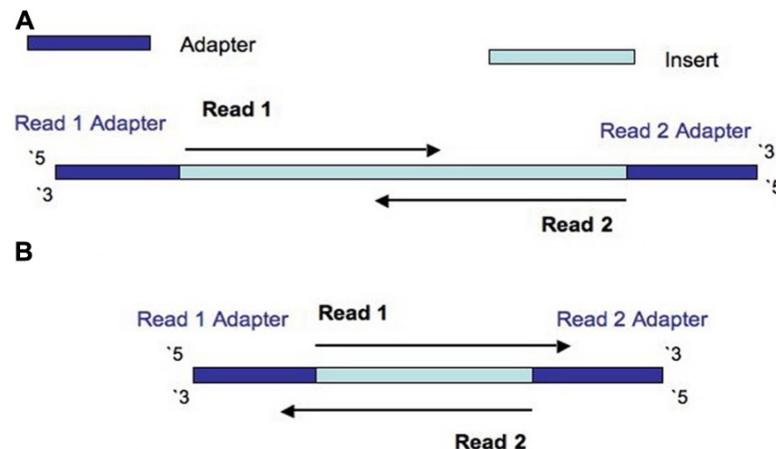
where p is an estimate of the probability that the alignment does not correspond to the read's true point of origin

- Mapping quality related to uniqueness
- Reads that map in multiple places can bias analysis

Mapping quality is not considered in estimation of genotype likelihoods in current software  
(so you may want to be conservative in filtering)

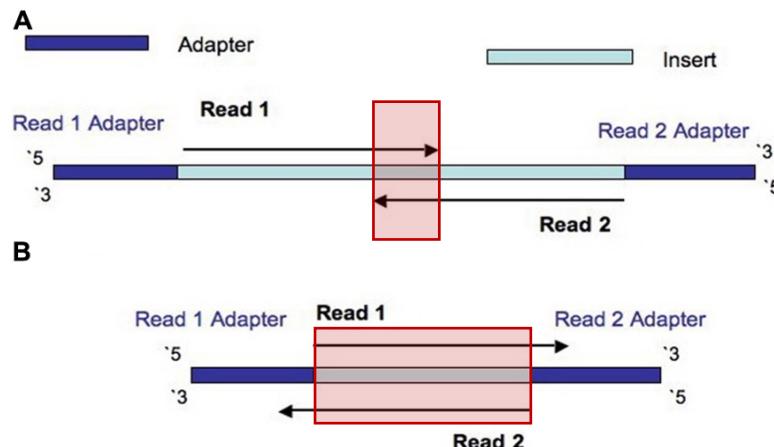


## Clip read overlap





# Clip read overlap



## Overlapping reads of the same DNA fragment

Avoid double-counting by soft-clipping the overlap



Merge all alignment files with sequence from the same library

- Reads from separate sequencing runs should be mapped separately to keep read group identifier
- But we need
  - A single bam file per library for deduplication
  - A single bam per individual for downstream analysis



## Remove duplicate sequence

- Duplicate reads are defined as originating from a single fragment of DNA
  - PCR duplicates (arise during library preparation)
  - Optical duplicates (arise during sequencing)
- Duplicates can bias our downstream analysis

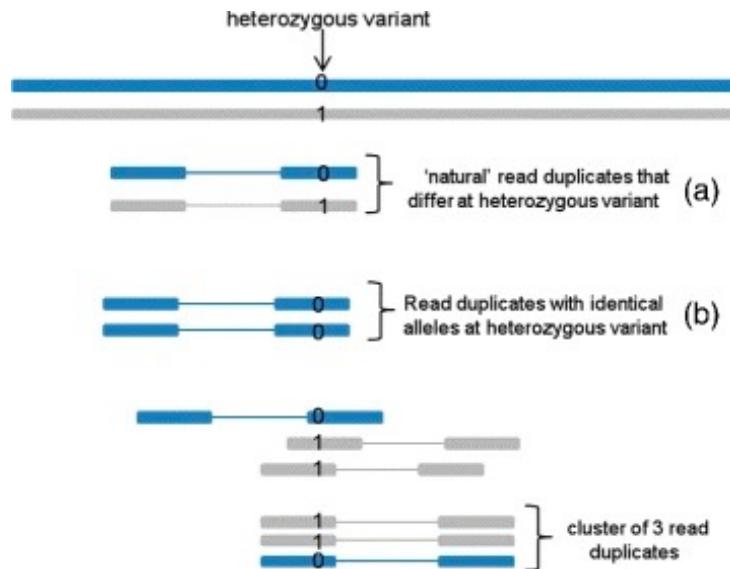
Filter alignments

Merge files

Deduplicate

Realign

## Remove duplicate sequence





## Realign around indels

- Genome aligners can only consider each read independently, and the scoring strategies they use to align reads relative to the reference limit their ability to align reads well in the presence of indels

example: 1000G Phasel low coverage  
chr15:81551110, ref:CTCTC alt:ATATA

Interpreted as 3 SNPs

Interpreted as microsatellite expansion/contraction

example: 1000G Phasel low coverage  
chr20:708257, ref:AGC alt:CGA

ref: TATAGAGAGAGAGAGAGAGC GAGAGAGAGAGAGAGGGAGAGACGGAGTT

alt: TATAGAGAGAGAGAGAGC GAGAGAGAGAGAGAGAGGGAGAGACGGAGTT

ref: TATAGAGAGAGAGAGAGAGC -- GAGAGAGAGAGAGAGGGAGAGACGGAGTT

alt: TATAGAGAGAGAGAGAG -- CGAGAGAGAGAGAGAGAGGGAGAGACGGAGTT



## Realign around indels

- Genome aligners can only consider each read independently, and the scoring strategies they use to align reads relative to the reference limit their ability to align reads well in the presence of indels
- Local realignment considers all reads spanning a given position.
  - Can more confidently infer indel polymorphisms
- Then all reads can be realigned around known indels

# General recommendations on filtering

But appropriate filters depends on the dataset and project – no one size fits all!

LOU ET AL.

MOLECULAR ECOLOGY WILEY 9

TABLE 3 Key data filters to consider in the analysis of IcWGS data

Category	Filter	Recommendation
General filters	Base quality	Base quality scores are factored into the calculation of genotype likelihoods, so if they accurately reflect the probability of sequencing error, bases with low scores also carry useful information. However, base quality scores are sometimes miscalibrated, so noise may be reduced if bases with scores below a threshold (e.g., 20) are either trimmed off prior to analysis or ignored. Alternatively, all base quality scores can be recalibrated based on estimated error profiles in the data (see Section 3.1).
	Mapping quality	Mapping quality is not considered in genotype likelihood estimation in currently available tools, so it is often advisable to remove low-confidence and/or nonuniquely mapped reads prior to analysis (e.g., reads with mapping quality <20). Filtering out reads that do not map in proper pairs should also further increase confidence in reads being mapped to the correct location, but could cause biases in regions with structural variation.
	Minimum depth and/or number of individuals	To avoid sites with low or confounding data support in downstream analysis, minimum depth and/or minimum number of individual filters can be used to exclude sites with much reduced sequencing coverage compared to the rest of the genome (e.g., regions with low unique mapping rates, such as repetitive sequences). Appropriate thresholds will vary between data sets, but could, for example, exclude sites with read data for <50% of individuals (globally or within each population), or with <0.8x average depth across individuals (after filtering on mapping quality)
	Maximum depth	Maximum depth filters are used to exclude sites with exceptionally high coverage (e.g., regions that are susceptible to dubious mapping, such as copy number variants). Common maximum depth thresholds could be one or two standard deviations above the median genome-wide depth.
Duplicate reads		PCR and optical duplicates can give inflated impressions of how many unique molecules have been sequenced. Duplicates can also lead to erroneous genotype calls, especially in heterozygous sites. Filtering out PCR and optical duplicates is recommended, but the threshold for filtering may need to be adjusted depending on the sequencing method and sample type.

# Now, your turn!

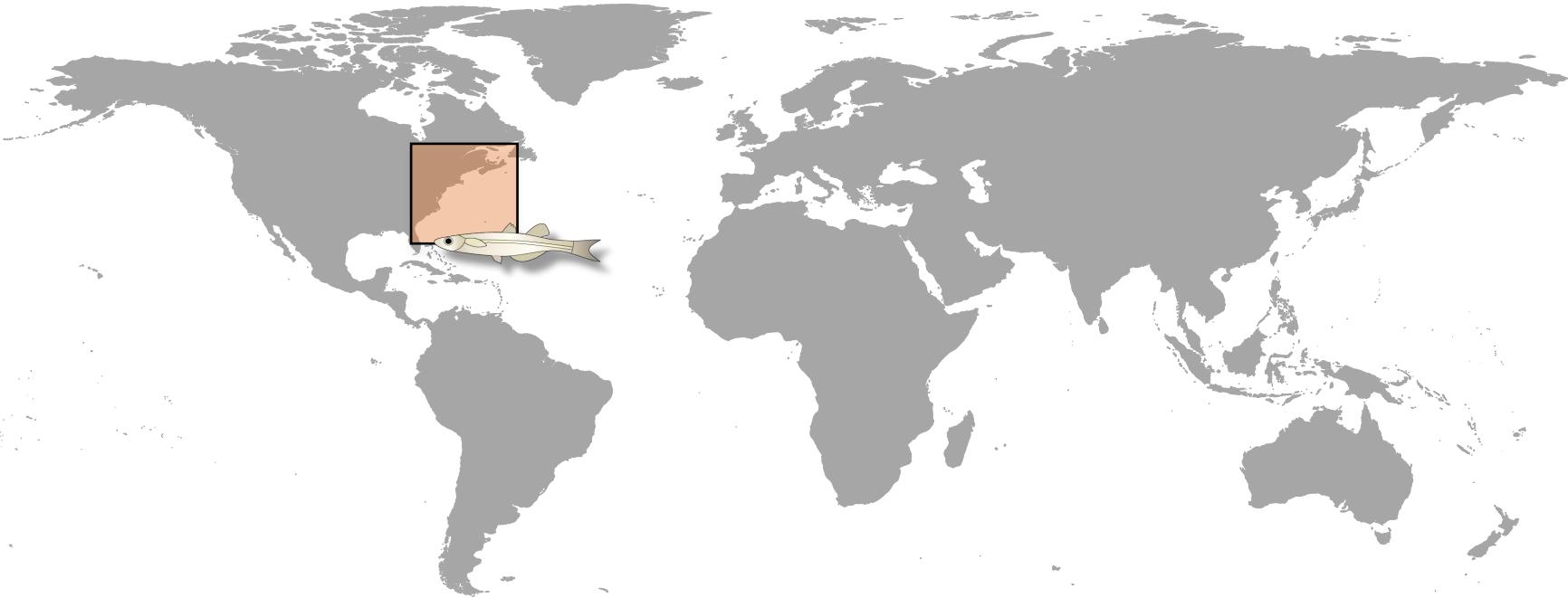
Example data for practicals

[https://github.com/nt246/physalia-lcwgs/blob/main/day\\_1/markdowns/data\\_processing.md](https://github.com/nt246/physalia-lcwgs/blob/main/day_1/markdowns/data_processing.md)

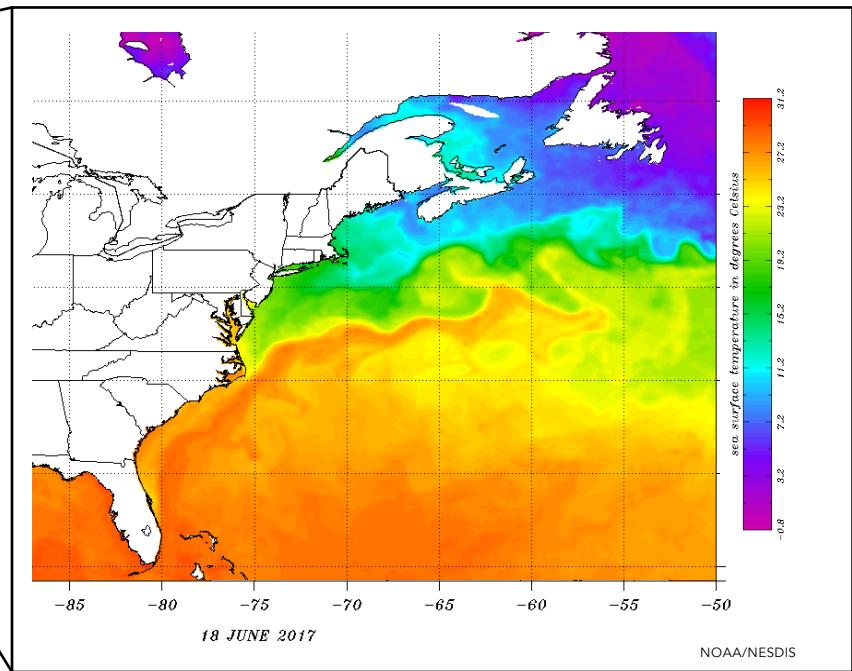
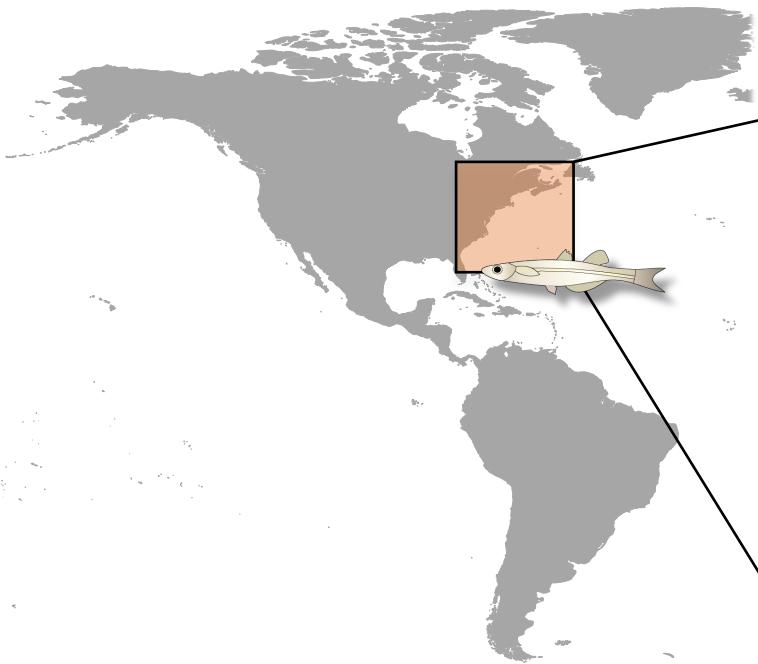
# Atlantic silverside *Menidia menidia*

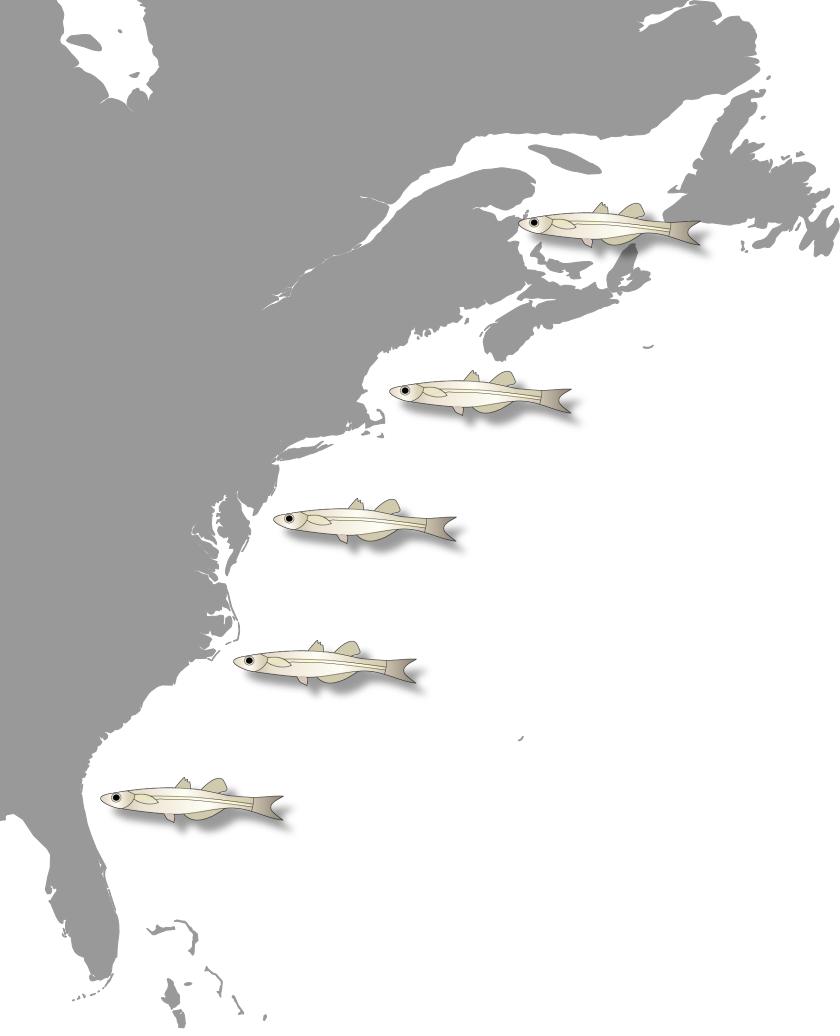


Photo: Jacob Snyder

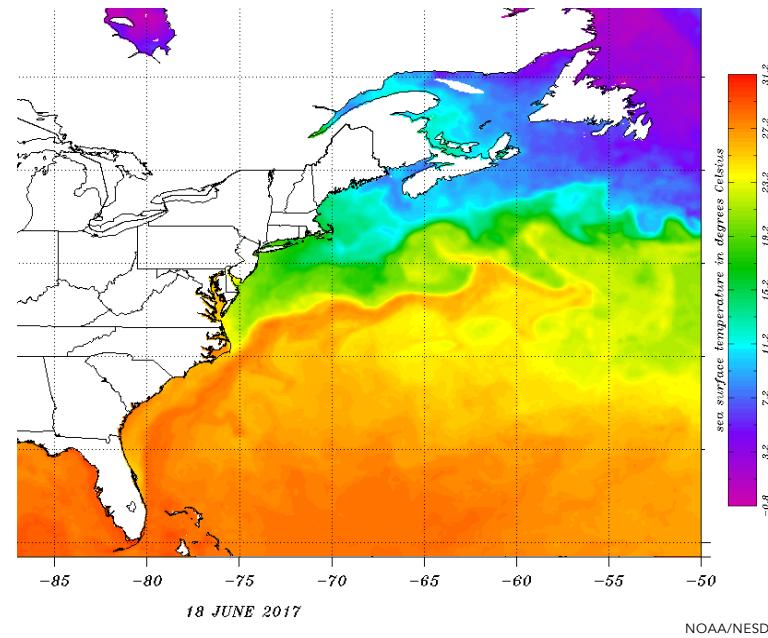


One of the world's steepest  
thermal gradients



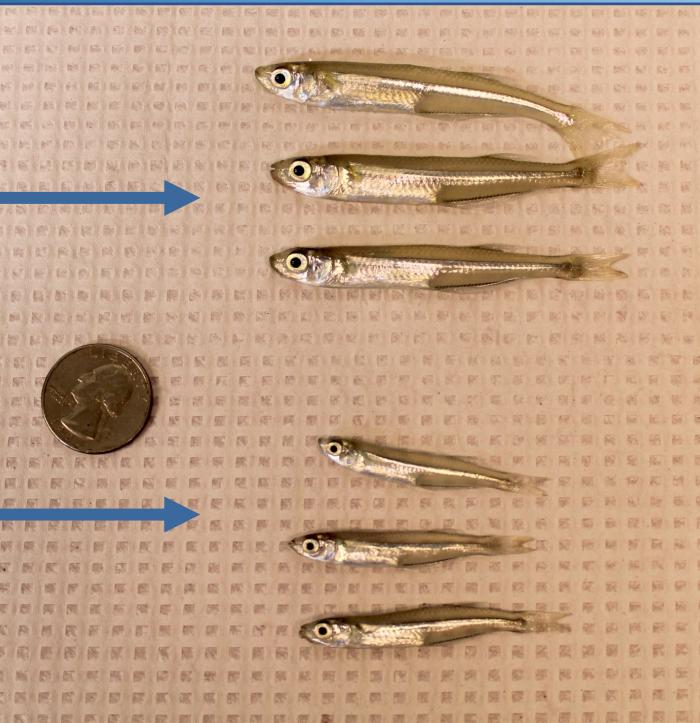


One of the world's steepest  
thermal gradients





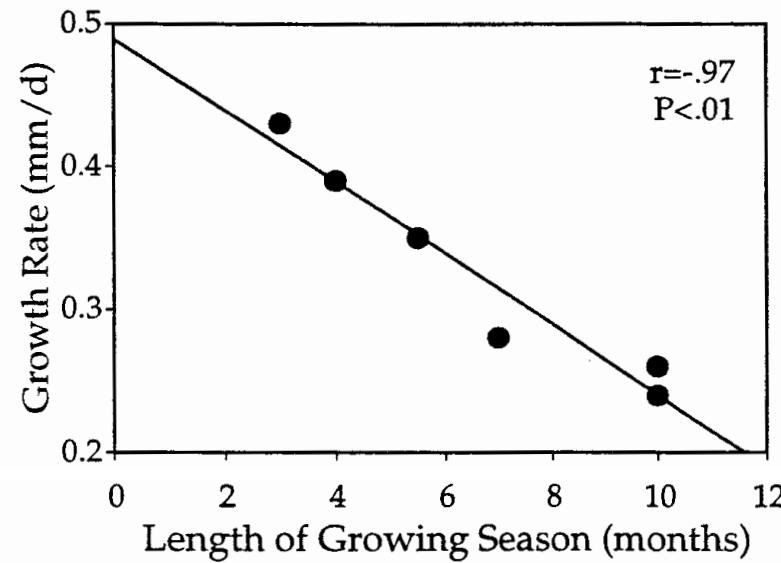
Same age,  
Common lab environment



# Growth capacity is tightly correlated with latitude

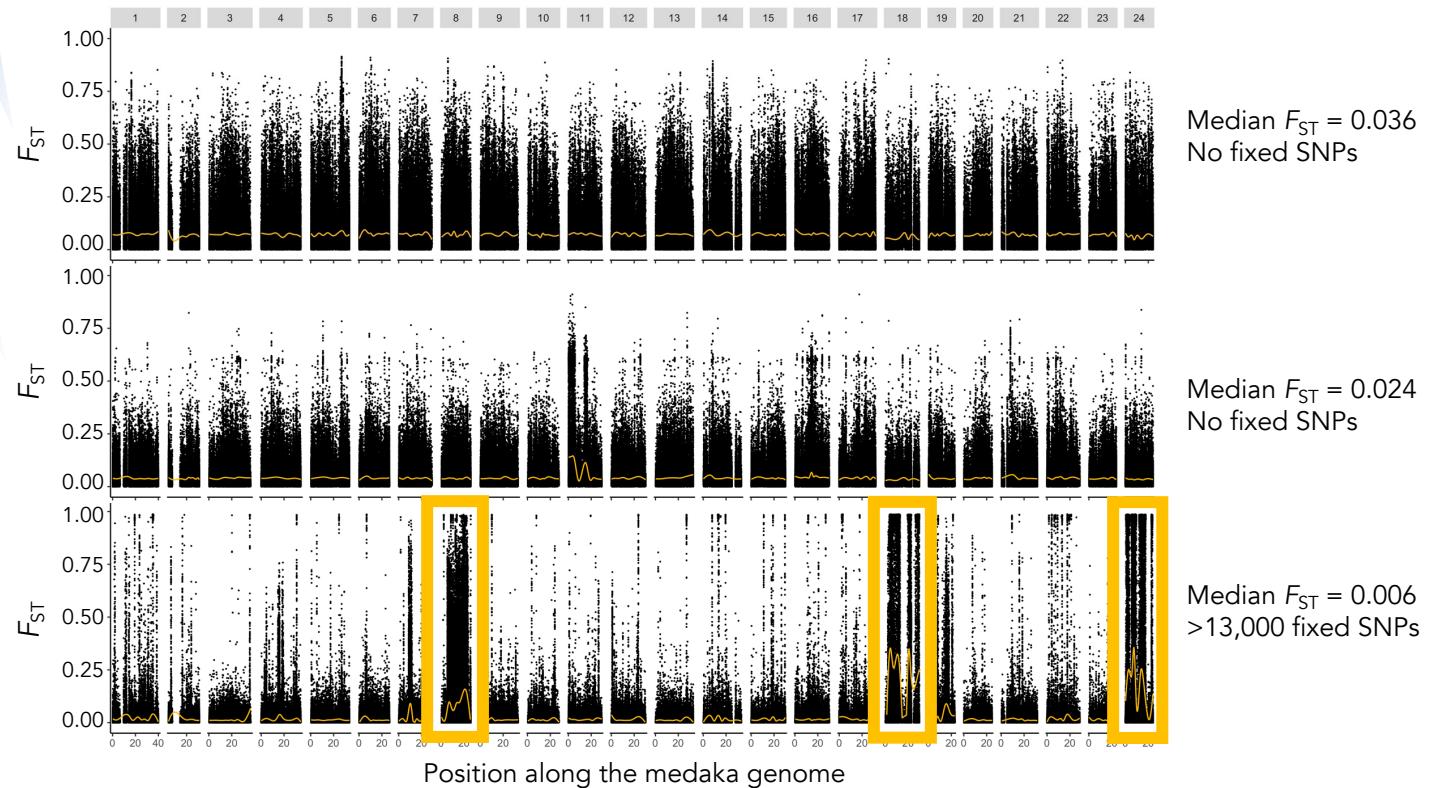
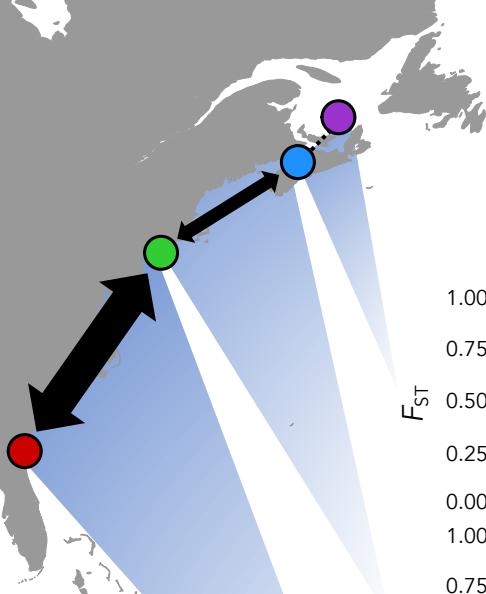


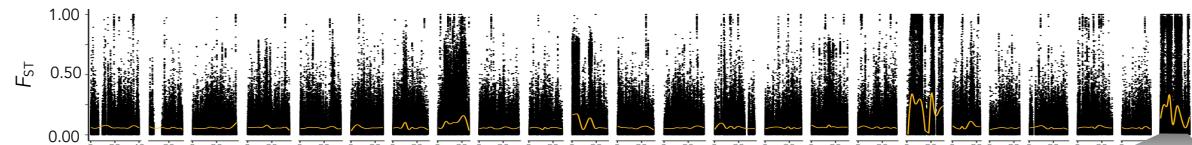
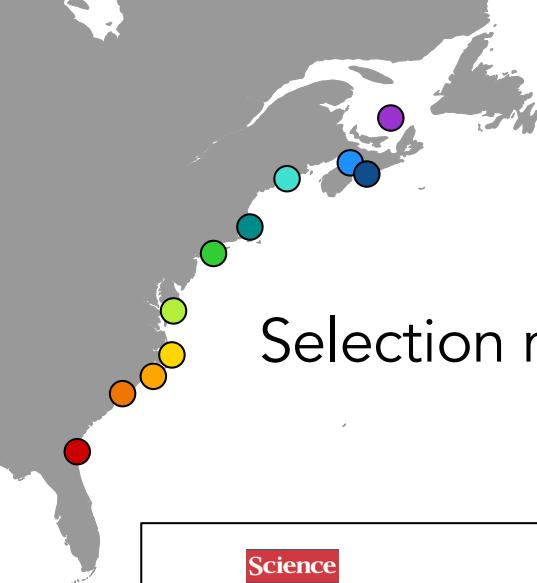
David Conover et al.



Reproduced from Conover. 1998. Bull. Mar. Sci.

# Heterogeneous gene flow and divergence patterns across latitudes





# Selection mapping through a fisheries experiment

**Science**  
AAAS

REPORTS

## Sustaining Fisheries Yields Over Evolutionary Time Scales

David O. Conover\* and Stephan B. Munch

Fishery management plans ignore the potential for evolutionary change in harvestable biomass. We subjected populations of an exploited fish (*Menidia menidia*) to large, small, or random size-selective harvest of adults over four generations. Harvested biomass evolved rapidly in directions counter to the size-dependent force of fishing mortality. Large-harvested populations initially produced the highest catch but quickly evolved a lower yield than controls. Small-harvested populations did the reverse. These shifts were caused by selection of genotypes with slower or faster rates of growth. Management tools that preserve natural genetic variation are necessary for long-term sustainable yield.

It is well established that wild pest and pathogen populations may evolve in response to anthropogenic forces of mortality (1), but is the same true of fisheries? Fishing mortality is highly selective. Exploited stocks typically display greatly truncated size and age distributions that lack larger and/or older individuals (2–4). This occurs not only because fishers may seek to exploit large individuals but also because regulatory measures often impose minimum size or gear regulations that ensure selective harvest of

on the basis of one of specific rules: (i) in two populations, more than the 10th percentile (the largest 90%) were harvested (i.e., large-harvested); (ii) in two other populations, less than the 90th percentile (the smallest 10%; small-harvested); and (iii) in two control populations, no harvest was imposed with respect to size (large-harvested controls). Survivors ( $n \approx 100$ ) were imaged and then randomly assigned to period manipulations to simulate fishing mortality. Lines were collected and rear-scaled at least once per generation under multiple growth conditions over four generations. Our methods in the supplementary material.

Cross-generation trends in harvested populations strongly supported our hypothesis (Fig. 1). Large-harvested populations initially produced the mean weight of fish but then decreased. By contrast, small-harvested populations initially produced the mean weight of harvested fish but then increased. By generation 4, the mean weight of harvested fish in large-harvested lines was nearly double that in small-harvested lines. Moreover, the spawning stock biomass differed even more. The mean weight of individual spawners (i.e., the survivors) in generation 4 was 1.05, 3.17, and 6.47 g in the large-, random-, and small-harvested

